Structure, Flexibility, And Overall Motion Of Transmembrane Peptides Studied By NMR Spectroscopy And Molecular Dynamics Simulations

by

Tyler Reddy

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

at

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DALHOUSIE UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

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Dedication

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To my parents (John & Sandra),  \text{my sister } (Rose\text{-}Marie),  and a
mazing friends from the Dalhousie & Halifax running communities.
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Abstract

Nuclear magnetic resonance (NMR) spectroscopy was used to determine the structure of transmembrane (TM) segment IX of the Na⁺/H⁺ exchanger isoform 1 (NHE1) in dodecylphosphocholine micelles. Studying isolated TM segments in this fashion constitutes a well-established "divide and conquer" approach to the study of membrane proteins, which are often extremely difficult to produce, purify, and reconstitute in full-length polytopic form. A similar approach was combined with NMR spin relaxation experiments to determine the peptide backbone flexibility of NHE1 TM VII. The combined NMR structural and dynamics studies are consistent with an important role for TM segment flexibility in the function of NHE1, a protein involved in apoptosis and myocardial disease. The study of the rhomboid protease system is also described from two perspectives: 1) I attempted to produce several TM constructs of the substrate spitz or a related construct and the production and purification are described in detail; and 2) I present coarse-grained molecular dynamics simulation results for the E. coli rhomboid ecGlpG and a spitz TM construct. Spitz appears to preferrentially associate with rhomboid near TMs 1 and 3 rather than the proposed substrate gate at TM 5. The two proteins primarily interact at the termini of helices rather than within the hydrocarbon core of the bilayer. Finally, I present a detailed analysis of coarse-grained molecular dynamics simulations of the fibroblast growth factor receptor 3 TM domain dimerization. Specifically, algorithms are described for analyzing critical features of wild-type and G380R mutant constructs. The G380R mutation is the cause of achodroplasia, the most common form of human dwarfism. The results suggest that the proximity of a residue to the dimer interface may impact the severity of the mutant phenotype. Strikingly, heterodimer and mutant homodimer constructs exhibit a secondary dimer interface which may explain the increased signaling activity previously reported for the G380R mutation—the helices may rotate with the introduction of G380R. The unifying theme of this work is the 'study of membrane proteins' using complementary techniques from structural biology and computational biochemistry.

List Of Abbreviations Used

ACN acetonitrile

AIC Akaike's information criteria

Boc tert-butoxycarbonyl

CD circular dichroism

CG coarse-grained

CG-MD coarse-grained molecular dynamics

cNHE1 cysteineless NHE1

CSI chemical shift index

DI-H₂O deionized water

DIEA N,N-diisopropylethylamine

DMSO dimethyl sulfoxide

DPC dodecylphosphocholine

DPPC dipalmitoylphosphatidylcholine

DSS (d_6) -2,2-dimethyl-2-silapentane-5-sulfonic acid

DTT dithiothreitol

EGFR epidermal growth factor receptor

ER endoplasmic reticulum

ESI electrospray ionization

FGF fibroblast growth factor

FGFR fibroblast growth factor receptor

FGFR3 fibroblast growth factor receptor 3

Fmoc 9-fluorenylmethoxycarbonyl

FRET fluorescence resonance energy transfer

GAF Gaussian axial fluctuation

GOBP general odorant-binding protein

GpA glycophorin A

HATU 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophos-

phate methanaminium

HBTU O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate

HPLC high performance liquid chromatography

HSQC heteronuclear single quantum correlation

IDP intrinsically disordered protein

Ig immunoglobulin

iRED isotropic reorientational eigenmode dyanmics

MALDI matrix-assisted laser desorption/ionization

MD molecular dynamics

MTSES (2-sulfonatoethyl) methanethiosulfonate

MTSET (2-(trimethylammonium) ethyl)methanethiosulfonate

NHE1 Na^+/H^+ exchanger isoform 1

NMR nuclear magnetic resonance

NOESY nuclear Overhauser effect spectroscopy

PARL presenilin-associated rhomboid-like

Pbf 2,2,4,6,7-pentamethyl-dihydrobenzofuran

POPC 1-palmitoyl-2-oleoyl-phosphatidylcholine

PPS propeptide subtilisin

RTK receptor tyrosine kinase

SNase staphylococcal nuclease

SPPS solid-phase peptide synthesis

tBu tert-butyl

TFA trifluoroacetic acid

TM transmembrane

 ${\bf TMD} \qquad \qquad {\bf transmembrane\ domain}$

TOCSY total correlation spectroscopy

Trt triphenylmethane

WT wild-type

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Drs. Michael Lumsden and Kathy Robertson, and more recently a 700 MHz spectrometer at the Biomolecular Magnetic Resonance Facility (BMRF—National Research Council Canada; Halifax, NS) has been employed. Many NMR experiments were performed at the Canadian National High Field NMR Centre (NANUC; Edmonton, AB) on an 800 MHz spectrometer with assistance from Dr. Ryan McKay. Operation of NANUC is funded by CIHR, NSERC, and the University of Alberta. Molecular dynamics simulations were performed using resources provided at the SBCB (Oxford, U.K.) and by ACEnet, the regional high performance computing consortium for universities in Atlantic Canada. ACEnet is funded by the Canada Foundation for Innovation (CFI), the Atlantic Canada Opportunities Agency (ACOA), and the provinces of Newfoundland & Labrador, Nova Scotia, and New Brunswick.

Chapter 1

Introduction

If there is a single unifying theme to this body of work it would have to be the 'study of membrane proteins.' Chapter 2 (page 3) concerns the nuclear magnetic resonance (NMR) spectroscopic based elucidation of the structure of the ninth transmembrane (TM) segment of the Na⁺/H⁺ exchanger isoform 1 (NHE1) in a membrane-mimetic medium. Studying an isolated TM segment is a well-established strategy to improve tractability by overcoming the difficulty of producing, purifying, and reconstituting polytopic membrane proteins. This "divide and conquer" approach (5) was also used to study the seventh TM segment of NHE1 as detailed in Chapter 3 (page 27), but in this case I examined the ps-ns and μ s-ms timescale dynamics of peptide backbone flexibility in a membrane-mimetic environment using NMR spin relaxation experiments. I employed the common Lipari-Szabo model-free analysis (6, 7) as well as reduced spectral density mapping (8, 9) to gain insight into the dynamics of the system. The following two chapters are based on three of my published manuscripts (1, 2, 3).

In Chapter 4 (page 58), I switch to the study of the rhomboid protease system. However, in keeping with the above theme, rhomboids are polytopic membrane proteins which cleave single-pass TM proteins within the phospholipid bilayer in prokary-otes and eukaryotes (10). My specific objective was the production, purification, and NMR-based structural study of a construct of the TM portion of the *Drosophila*

spitz peptide—part of the natural substrate of Rhomboid-1. This is a labwork-heavy chapter as it was extremely challenging to produce and purify a number of different spitz-based or related constructs.

Although NMR-based structural and dynamics studies of membrane proteins can provide a lot of valuable information, one missing piece of information is how the peptide or protein structures move (on a larger scale) within phospholipid bilayers and interact with other proteins. I have employed coarse-grained molecular dynamics (CG-MD) simulations to study protein-lipid and protein-protein interactions for two membrane protein systems. In chapter 5 (page 108), the dimerization of the fibroblast growth factor receptor 3 (FGFR3) TM domain (of particular interest because of its role in human achondroplasia (*i.e.*, reference (11))) is studied in detail using CG-MD. This chapter integrates a detailed discussion of analytical algorithms used to parse crucial information from wild-type homodimer, heterodimer, and mutant homodimer FGFR3 simulation trajectories. Chapter 6 (page 191) outlines preliminary CG-MD simulation results for a system consisting of a rhomboid protease construct and a spitz TMD construct in a phospholipid bilayer.

This work therefore employs complementary techniques from structural biology and computational biochemistry to probe a number of membrane protein systems. The self-contained introductions in subsequent chapters will introduce the specific systems and techniques in detail, and the appendices contain documented source code and additional results.

Chapter 2

NMR Structure Of NHE1 TM IX (Based On Published Manuscript (1))

2.1 Introduction

The human Na⁺/H⁺ exchanger isoform 1 (NHE1) is involved in a number of important biological processes. These include regulation of intracellular pH, cell growth and differentiation, cell migration, and regulation of sodium fluxes (reviewed in (12)). There is also substantial interest in NHE1 because of its established role in the myocardial damage that occurs during ischemia, reperfusion and a possible role in mediating cardiac hypertrophy. Despite the motivations for studying NHE1, there are no high-resolution atomic structures of the protein available in the literature.

NHE1 is a ubiquitously expressed plasma membrane protein, and based on functional and phylogenetic analysis it is proposed to include 12 N-terminal TM segments and a C-terminal regulatory domain (13, 14). Our objective was to use an NMR spectroscopy-based approach to obtain high-resolution atomic structural information on NHE1. In light of the poor tractability of the polytopic TM protein, we opted to use the "divide and conquer" approach to membrane protein structure determination (reviewed in (5)). This method consists of producing isolated TM segments, solving their structures in membrane-mimetic environments, and then recombining the

individual TM segment structures to get an overall picture of the polytopic configuration. The NMR structures of a number of NHE1 TM peptide segments have been solved as part of a "divide and conquer" strategy, and I have described the details of these structures (along with several successful examples of the "divide and conquer" approach) in the published manuscript (1).

We specifically chose to study NHE1 TM segment IX (residues 339-363 in the Wakabayashi topology (13)) for three reasons: 1) TM IX is important in mediating sensitivity to NHE1 antagonists (15); 2) Alteration of H349 in TM IX reduced exchanger sensitivity to amiloride (inhibitor) compounds (16); 3) Site-directed mutagenesis is consistent with additional importance for TM IX in NHE function and drug sensitivity (17, 18).

My work on a 31-residue synthetic peptide construct of NHE1 TM IX employed circular dichroism (CD) spectropolarimetry and NMR spectroscopy for structural characterization. In parallel, our collaborators in the laboratory of Dr. Fliegel (University of Alberta) conducted a cysteine-scanning mutagenesis study to determine the importance of each residue in TM IX for full-length cysteineless NHE1 (cNHE1) activity. The latter functional methods and results are detailed in the manuscript (1), while the structural results are described below with integration of crucial functional results where appropriate.

2.2 Materials And Methods

2.2.1 Materials

Deuterated dodecylphosphocholine (DPC) was purchased from C/D/N isotopes (Pointe-Claire, Quebec, Canada).

2.2.2 Peptide Synthesis And Purification

A (> 95 %) purified 31-residue peptide construct of NHE1 TM IX containing

cationic caps at the N- and C- termini (KSYMAYLSAELFHLSGIMALIASGVVMRPKK; acetylcapped N-terminus, amide-capped C-terminus) was purchased from GL Biochem (Shanghai, China). Purity was assessed by high performance liquid chromatography (HPLC) and identity was confirmed by matrix-assisted laser desorption ionization (MALDI) mass spectrometry and sequential assignment of NMR spectra.

2.2.3 NMR Spectroscopy And Structure Calculations

An NMR sample was prepared by dissolving 0.9 ± 0.1 mM peptide (concentration estimated from ¹H one-dimensional NMR spectrum integrations relative to the internal standard using assigned resonances) in 95% H₂O, 5% D₂O solution containing ~ 75 mM deuterated DPC and 0.25 mM (d_6)-2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) for chemical shift referencing. The experiments were conducted at 30 °C on the sample with pH 5.05 (without accounting for deuterium isotope effects). One-dimensional ¹H, natural abundance gradient-enhanced ¹H-¹³C HSQC (heteronuclear single quantum correlation), two-dimensional ¹H-¹H TOCSY (total correlation spectroscopy) (60-ms mix; decoupling in the presence of scalar interactions spin lock), and NOESY (nuclear Overhauser effect spectroscopy) (225-ms mix) experiments were acquired on the Canadian National High Field NMR Centre Varian INOVA 800-MHz spectrometer. All experiments were used as configured within the Varian BioPack software package. Spectra were processed using NMRPipe (19) and analyzed using Sparky 3 (20). All spectral assignments were carried out by manual peak picking in Sparky, and resonance assignments have been deposited in the BioMagResBank (code 15747).

Structure calculations were performed in the Python scripting interface of XPLOR-NIH version 2.18 (21) using NOE restraints from the assigned spectrum. Peak volumes were independently calculated using Gaussian and Lorentzian fit Sparky algorithms, with no allowed peak center motion. Those peaks that were not assigned a volume or were assigned a negative volume by the algorithm (~27.5%) were either manu-

ally assigned a summed signal intensity as defined by a user-specified region or fit by Sparky algorithm after contour adjustment. Peak volumes were empirically calibrated to distance ranges of 0-5.0, 1.8-5.0, and 1.8-6.0 Å; the Lorentzian fit volumes were more conservative and used for subsequent analysis. Restraints were divided into bin ranges corresponding to strong (1.8-2.8 Å), medium (1.8-4.0 Å), weak (1.8-5.0 Å), and very weak (1.8-6.0 Å) contacts. Ambiguous assignments and NOE potential scaling were handled as described previously (22). Following 21 rounds of structural refinement by simulated annealing, an additional 6 rounds were performed with dihedral angle potential scaling factors of 5, 50, 100, 100, 50, and 25. Simulated annealing parameters were similar to those described previously (22), but with 15000 cooling steps.

A single extended polypeptide was generated and subjected to simulated annealing for each round. To handle the families of 100 structures generated per round an in-house tcl/tk script (freely available upon request from J. Rainey) was used; the script allowed for assessment of violations and iterative refinement of NOE restraints. Refinement gradually increased in stringency, initially violations > 0.5 Å in > 50 % of structures were lengthened, but subsequently violations > 0.1 Å in > 25 % of structures were modified. After 21 cycles of simulated annealing and NOE refinement, calculated XPLOR-NIH structure energies contained minimal contributions from NOE violations, and magnitudes of all observed violations were minimal. All NOE restraints were satisfied without pruning. Six further cycles of simulated annealing were carried out with incorporation of dihedral angle restraints as described above. The lowest 40 energy structures in the final ensemble of 100 were retained for further analysis. The final sets of restraints have been deposited in the Protein Data Bank with this ensemble of 40 structures (Research Collaboratory for Structural Bioinformatics Protein Data Bank code 2k3c).

2.2.4 CD Spectroscopy And Analysis

Samples for CD spectroscopy were diluted from the previously described NMR sample to obtain samples of ~10 μ M peptide, ~3 mM deuterated DPC, and the unbuffered pH was adjusted to ~4.8 in a chloride-free solution. Nine replicate measurements were collected over 3 separate days on a Jasco J-810 spectropolarimeter (Easton, MD) at 30 °C in a 0.1-cm path length (Hellma, Müllheim, Germany) water-jacketed cell (20 nm/min scan rate). Data were collected in a wavelength range of 260-180 nm but the signal to noise ratio was considered reasonable only at a detector (photomultiplier tube) voltage under 700 V (based on previous instrumental observation). Averaged data were analyzed by several algorithms (23) using the DICHROWEB interface (24, 25).

2.3 Results

2.3.1 Peptide Design And Conditions For NMR Spectroscopy

The synthetic peptide contains three lysine residues, one at the N terminus and two at the C terminus, which are not present in the endogenous sequence of the NHE1 protein. Cationic residues at the termini of peptides have been shown to facilitate both peptide purification and maintenance of transbilayer orientation (26). For convenience, the lysine residues are numbered relative to the endogenous sequence. All structural studies were conducted on the 31-residue peptide produced by chemical synthesis with no isotope labels.

TM peptide solubility is often problematic, and while NHE1 TM IV was soluble in organic solvents (27), TM VII was substantially more soluble in DPC micelles (22). We opted to use DPC micelles for solubilization of TM IX because this system is proposed to be a more appropriate membrane-mimetic than organic solvents (28). Sample components were 0.9 ± 0.1 mM peptide, ~75 mM deuterated DPC, and 0.25 mM DSS in 95% H₂O, 5% D₂O adjusted to pH ~5.05 and studied at 30 °C. This

temperature provided good NMR spectral characteristics, prevented precipitation for extended periods of spectroscopic study, and allows for use of the cryogenically cooled triple-resonance probe on the 800-MHz Canadian National High Field NMR Centre spectrometer. This combination of factors allowed determination of the structure of the TM IX segment in DPC micelles.

2.3.2 Resonance Assignment And Structure Calculation

Sequential chemical shift assignments were carried out using two-dimensional TOCSY, natural abundance ¹H-¹³C HSQC, and NOESY experiments (22, 29) (Figure 2.1 on page 17). Acquisition of a natural abundance ¹H-¹⁵N HSQC data set was not feasible because of low signal-to-noise arising from the tumbling rate of the peptide-containing micelles. Resonance assignments for ¹H were complete except for Met-H $^{\epsilon}$ and Phe-H $^{\delta}$. In some cases there were missing or ambiguous 13 C assignments $\text{for Tyr-C}^{\alpha}, \ \text{Met-C}^{\alpha}/\text{C}^{\epsilon}, \ \text{Leu-C}^{\beta}/\text{C}^{\gamma}/\text{C}^{\delta 1}, \ \text{Ser-C}^{\alpha}/\text{C}^{\beta}, \ \text{Glu-C}^{\alpha}, \ \text{Phe-C}^{\beta}, \ \text{His-C}^{\alpha}/\text{C}^{\delta 2}, \\ \text{Ser-C}^{\alpha}/\text{C}^{\beta}, \ \text{Clu-C}^{\alpha}, \ \text{Clu-C}^{\alpha}, \ \text{Clu-C}^{\beta}, \ \text{Clu-C}^{\alpha}, \ \text{Clu-C}^{\beta}, \ \text{Clu-C}^{\beta},$ and Lys- C^{γ} . Assessment of unambiguous assignments was made as described previously (22). The latter approach involves the use of ambiguous assignments in structure calculations where unambiguous assignments were not possible. A total of 1231 unique NOE restraints (Table 2.1 on page 25) were used for calculation of the TM IX structure. These are summarized graphically in terms of the standard connectivities examined for secondary structure characterization (Figure 2.2 on page 18) and in terms of the number of unique restraints per residue (Figure 2.3 on page 18). A progressive energy minimization of calculated NMR structural ensembles with each round of refinement is detailed in Figure 2.4 on page 19.

2.3.3 Structural Analysis Of TM IX Peptide

An ensemble of the 40 lowest energy structures from 100 calculated peptide structures was obtained that satisfy the 1231 observed unique NOE restraints with minimal violations (Table 2.1 on page 25). Dihedral angle restraints ($\phi = -60 \pm 30$;

 $\psi = -40 \pm 40$) consistent with α -helical structure were imposed over residues M340-S344 and I353-S359, based on the seconday chemical shift analysis (Figure 2.5 on page 20). The dihedral restraints resulted in a significant but reasonable (~17 %) inflation in total energy relative to an ensemble restrained only by NOEs (Table 2.1 and Figure 2.4).

A 9-replicate averaged CD spectrum was analyzed using the DICHROWEB (24, 25) interface by applying several secondary structure deconvolution algorithms (results summarized in Table 2.2 on page 26). A 190 nm low-wavelength cutoff was used for meaningful signals as detailed in section 2.2.4. The normalized standard deviation parameter was used to filter those algorithms that did not produce a good fit to the data (normalized rmsd > 0.2) (30). The calculated average secondary structure contributions are 26 ± 5 % helix, 31 ± 6 % sheet, 16 ± 4 % turn, and 29 ± 10 % random (Table 2.2). In light of the relatively high average deviations across algorithms, a qualitative inspection of the CD spectra is relevant (Figure 2.6 on page 21). To reflect the 12/31 (~ 39 %) helical residues predicted based on NMR dihedral restraints, a (theoretical) 39 % helix CD spectrum from 200 to 240 nm was generated by K2D (23). The experimental curves are qualitatively consistent with a strong helical contribution.

Superposition of all members of the ensemble over the full length of the peptide was not possible. However, based on rmsd analysis as detailed in (29), K337-L350 and G352-V362 were respective N- and C-terminal segments consistent with a relatively invariant structural fold (Figure 2.7 on page 22). The intervening S351 serves as a pivot point between the N- and C-terminal portions of the peptide, although its dihedral angles across the ensemble of structures are well clustered (Figure 2.8 on page 23), and its dihedral angle order parameters are close to unity, but in close proximity to a flexible region (Figure 2.9 on page 24). Residues assigned to the two helical segments were also well clustered by dihedral angle (Figure 2.8). While the

penultimate C-terminal K residue was surprisingly well-clustered by dihedral angle, M363 (also near the C-terminus) had a large dispersion of backbone dihedral angles over the ensemble (Figure 2.8).

2.3.4 Structural Superposition Of TM IX

Peptide segments between 4 and 19 residues long were iteratively superimposed to provide a minimum rmsd relative to the backbone of the lowest energy structure (Figure 2.7 on page 22). Those superpositions producing the largest contiguous segments of residues with rmsd values < 1.0 were additionally filtered based on average rmsd across the entire segment. The following permutations were assessed: N-terminal segments between residues 337-342 and 345-354 and C-terminal segments between residues 349-354 and 357-367 were superimposed by the method of Kabsch (31) as implemented in the LSQKAB software of the CCP4 suite (32). K337-L350 and G352-V362 were the respective N- and C-terminal regions with rmsd values most consistent with a fixed region of structure (Figure 2.7 on page 22). The dihedral angle order parameter (Figure 2.9) was consistent with a region of structural flexibility (i.e., a pivot point at S351) between the determined segments.

2.4 Discussion

2.4.1 Structural Analysis Of TM IX

Where the full-length structure of proteins is available, studies have shown that isolated TM segments both reflect the structure of intact proteins and often retain their functional characteristics. Specific examples include bacteriorhodopsin (33, 34), rhodopsin (35), the cystic fibrosis TM conductance regulator (36, 37), and the fungal G-protein-coupled receptor Ste2p (38). Stabilization of the physiological structure of a TM segment may be solvent-dependent as observed previously with bacteriorhodopsin (33, 34), and TM IV (27) and TM VII (22) of NHE1. For this reason, we solubilized

the 31-residue synthetic TM IX peptide in membrane-mimetic DPC micelles, a system that has been well established (39, 40). We found that the structure of the TM IX peptide in DPC micelles is an interrupted helix with a sharp, although potentially flexible, bend immediately N-terminal to S351 (Figure 2.7 on page 22). This bend results in a kinked, "L"-shaped structure retained across amino acids 338-365. The relative position for the N- and C-terminal superposition segments around the S351 pivot is slightly variable across the ensemble. We have previously reported a similar observation for NHE1 TM VII in DPC micelles, which was also determined by NMR spectroscopy to be an interrupted helix, although with more variability in the angle of the bend between converged segments (22). The residues involved in the kink of TM VII were observed to be functionally critical residues. Similarly, it has been reported that helix IV of NHE1 is kinked at functionally important amino acids (27). The actual degree of flexibility in the context of the full-length protein would depend on constraining interactions arising from other helices, from the surrounding lipid bilayer, and through homodimer contacts (41). Kinked helices are thought to play an important role in transport function for the prokaryotic Na⁺/H⁺ exchanger NhaA (42), and the trend of studies on TM segments of NHE1 shows residues appearing at kinked locations that are functionally critical.

There are two different membrane-spanning topologies for the NHE1 protein proposed in the literature. The first, by Wakabayashi and co-workers (13), is based on cysteine-scanning accessibility analysis and led to the present widely used model with TM IX including amino acids 338-360. More recently a model was developed based on fold recognition using the *E. coli* NhaA crystal structure as a template for phylogenetic analysis and homology modeling (14). In this model, amino acids 338-360 of TM IX correspond to part of TM VII, a subsequent extracellular loop, and all of TM VIII. Landau *et al.* (14) suggest that the previously described TM IX is an artifact of the window size used for hydropathy analysis. However, the TM VIII arising from

their model has a membrane-spanning length of ~ 19 Å, which is short for a eukaryotic membrane (43).

2.4.2 Structure-Function Correlation

In addition to the structural studies described above, a complementary set of functional experiments on full-length cNHE1 in living cells were conducted by researchers in the laboratory of our collaborator (Dr. Fliegel, University of Alberta), and the results are detailed in the published manuscript (1). In all cases, they were able to express an intact Na⁺/H⁺ exchanger protein. However, five of the mutants had decreased NHE1 protein activity. For L343 and S351 this was due, at least in part, to decreased expression and improper targeting. For Y339, I353, A355, and V361, significant defects in protein function were introduced by mutation. The E346, I353, and V361 side chains face the opposite direction of Y339, S351 and A355 side chains in our structure (Figure 2.7). This would seem to conflict with their importance in function; however, Landau et al. (14) suggested that S351 faces the lipid bilayer despite its functional importance and that it can rotate by 180° to face the pore for ion coordination. If true, the segment may sometimes face the pore and sometimes the lipid bilayer.

Only two mutants, E346C and S351C, were strongly inhibited by reaction with MTSET ((2-(trimethylammonium) ethyl)methanethiosulfonate) (a positively charged sulfhydryl-reactive reagent), and no mutants were inhibited by MTSES ((2-sulfonatoethyl) methanethiosulfonate) (a negatively charged sulfhydrl-reactive reagent). The most likely explanation for their reactivity with MTSET is an interaction at a site that lines and blocks the ion translocation pore (44). There were minor inhibitory effects of MTSET on the adjacent amino acids A345 and L350 (1). Their mutation could cause a minor perturbation of the structure of TM IX in this region that affects E346 and S351 but is also consistent with these residues having at least partial exposure to the pore. In support of this concept, A345, E346, and L350 all cluster with side chains

on the same face of the segment throughout the ensemble of structures. Although S351 is consistently (37/40 ensemble members) on the opposite face of the peptide, this observation may be explained by the rotation mechanism suggested by Landau *et al.* (14).

Positively charged MTSET but not negatively charged MTSES inhibited NHE1 activity in the E346C and S351C mutants. This contrasts with the results for the F161C mutant of TM IV that was inhibited by both compounds (27) but mirrors results with TM VII (45). MTSET may disrupt cation translocation by direct electrostatic repulsion, whereas negative MTSES would not. Alternatively, MTSES may not be able to react with these amino acids because of repulsion on the protein surface from negatively charged amino acids important in ion coordination (46). Local protein conformation and chemistry have been shown to affect accessibility to sulfhydrylreactive reagents in both K⁺ channels (47) and in the FMRF-amide-activated sodium channel (48). The E346C cNHE1 mutant exhibited a higher degree of kinetic inhibition for the same measured parameters as S351C (1) and was similarly affected by MTSET (inhibition) and MTSES (no inhibition). In both topology models (13, 14) S351 is located in the intramembrane region of the protein. Therefore, our results are consistent with S351 being pore-lining in both cases and MTSET obstructing the pore. In the first topology model (13), E346 is also in the intramembrane region and should be pore-lining, potentially on the same face of a TM segment as S351. However, our structural analysis did not place these residues on the same face, and only 3/40 ensemble members show this positioning. This may suggest an extracellular loop position for E346, as proposed by Landau et al. (14). MTSET modification could then obstruct cation transport by preventing entry to the pore (E346).

The observed increase in $K_{m(Na^+)}$ for S351C mutants (in kinetic studies by our collaborators detailed in the published manuscript) is consistent with at least moderately reduced extracellular coordination efficacy, and may therefore be supportive of

a role in the extracellular cation funnel for S351 as suggested by Landau *et al.* (14). The decrease in $V_{max(Na^+)}$ may imply a measurable contribution to inhibition by a perturbation in overall pore structure or that ion coordination by S351 is rate-limiting (*i.e.*, that we are not simply observing reduced electrostatic interaction that can be overcome by ligand saturation). Finally, the reduced V_{max} values for H⁺ transport and especially the reduced intracellular (*i.e.*, allosteric) activation of S351C cNHE1 by H⁺ are in agreement with an overall perturbation in tertiary structure.

Based on dihedral restraints and rmsd analysis, our data suggest that M340-S344 is helical, and although this is consistent with these residues lining a TM segment as they do in both models, the lack of contiguous α -helical character observed in the ensemble C-terminal to this segment until the second major helical segment (I353-S359) would be consistent with the extracellular loop assignment of residues 345-352 (14). The observed pivot at S351 (Figure 2.7 on page 22) also supports the possibility of an adjacent flexible extracellular loop at the location suggested by Landau *et al.* (14). E346 and G352 have been implicated as being critical in NHE1 inhibitor binding (17, 18). Placement of these residues at or near the extracellular surface is consistent with a site of drug interaction.

A distorted and interrupted helix with a bent L-shape would result if the peptide NMR structure was treated as a single TM segment (13). The bent structure we observe is consistent with a eukaryotic membrane span, the distance between C^{α} of S338 and S359 is 28 ± 2 Å in our structures. Conversely, if the Landau *et al.* (14) topology is correct, residues H349 and V362 would be at the extracellular and intracellular faces, giving a transbilayer length of 19 ± 1 Å, a length typically assumed to be insufficient to span a eukaryotic membrane (43). To attempt to resolve these topological issues, our collaborators performed a series of experiments with amino acids 345-348 to determine the accessibility to the extracellular surface. However, it was not possible to get consistent results that either verified or disproved their

extracellular or TM location (not shown). Even when externally accessible residues are detected, they are sometimes assigned intracellular locations using the argument that they represent pore-lining residues (13), making assignment of location somewhat arbitrary. The functional studies (by our collaborators) showing inhibition by MTSET or our structural studies on the isolated TM IX peptide provided valuable data on the importance of these residues in function, but they are unable to shed light on their precise topology.

We found that amino acid M363 displayed an elevated dispersion of backbone dihedral angles, and this may suggest functionally important flexibility at this position (Figure 2.8 on page 23). However, the functional work of our collaborators showed that the M363C mutation did not eliminate activity and that it did not react with MTSET or MTSES (1), which is somewhat contradictory to the idea that it is critical to function. Wakabayashi et al. (13) suggested that M363C is in an extracellular loop. Landau et al. (14) placed M363 in an intracellular loop. Our results strongly support M363 as a loop residue displaying a high degree of flexibility, but they cannot localize this residue to either face of the membrane nor assign a critical pore-lining role to this amino acid.

Overall, the combination of my NMR structural studies with the work of our collaborators provides a detailed structural and functional picture of the functionally critical amino acids 339-363 of the NHE1 isoform of the Na⁺/H⁺ exchanger. Our collaborators demonstrate that 5 of 25 residues are very sensitive to mutation to cysteine, and that E346 and S351 are involved in cation translocation and likely line the cation pore (1). The ensemble of structures, representing a kinked helical peptide, is similar to that previously reported for TM VII (22), but with a larger bend angle at the pivot point, S351. Amino acids M340-S344 and I353-S359 are helical. To resolve whether amino acids 339-363 represent one TM segment or parts of two, a full-length structure of NHE1 is necessary. It is encouraging that expression and purification

of full-length NHE1 has been achieved, but currently only a low-resolution structure obtained by electron microscopy is available (41).

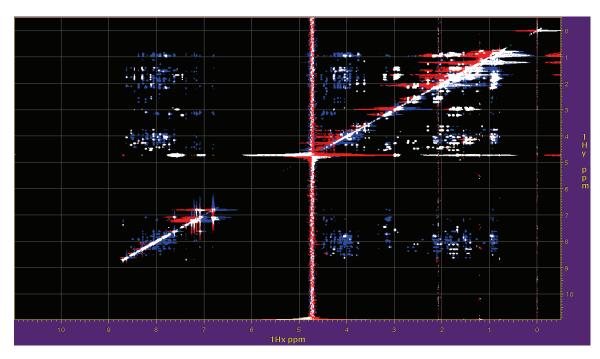


Figure 2.1: The NHE1 TM IX construct in DPC micelles was studied by TOCSY (white peaks) and NOESY (blue peaks) NMR experiments. There are substantially more peaks in the ¹H-¹H NOESY (225-ms mix) experiment than in the ¹H-¹H TOCSY (60-ms mix; decoupling in the presence of scalar interactions spin lock) on an 800 MHz spectrometer. This is consistent with the detection of through-space interactions in the NOESY and the restriction to scalar correlations in the TOCSY.

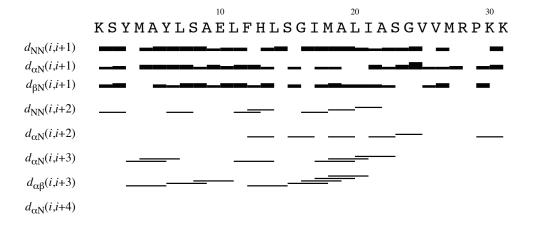


Figure 2.2: A representation of NOE contacts, which are limited to internuclear distances of ≤ 6 Å, between residues in the context of the primary sequence of NHE1 TM IX. This representation is modified from CYANA (L.A. Systems, Inc.) output and is consistent with the contacts outlined in Figure 2.3 on page 18 (*i.e.*, consider the paucity of long-range NOE restraints).

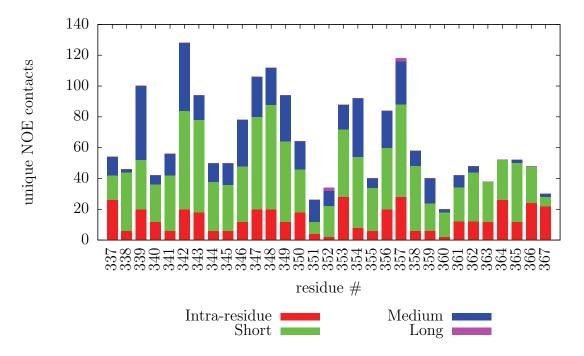


Figure 2.3: The number of per-residue unique NOE contacts in the final set of NMR restraints for NHE1 TM IX is summarized in this stacked histogram modified from CYANA (L.A. Systems, Inc.) output. Medium range restraints vary between 2 and 4 positions in the primary sequence, while long range restraints are five or more residues apart.

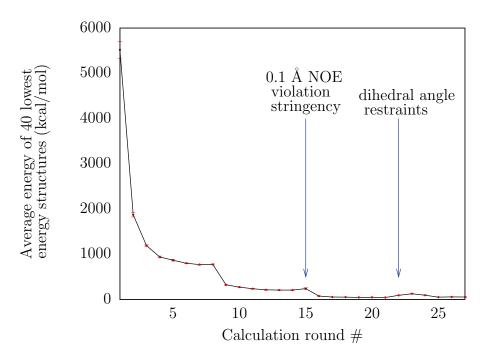


Figure 2.4: Tracking the total energy from each of the 27 rounds of XPLOR-NIH structure calculations for NHE1 TM IX. After each round, the average energy and standard deviation of the 40 lowest energy structures (out of 100 produced) were calculated. Iterative refinement of NOE restraints progressed by allowing restraints with violations > 0.5 Å in > 50% of structures to be lengthened, and after round 15 restraints with violations > 0.1 Å in > 25% of structures were lengthened. The latter progression in NOE restraint stringency clearly resulted in a reduction in average total energy for the calculated ensemble. After round 21, dihedral angle restraints consistent with α -helicity were imposed over candidate helical residues based on secondary chemical shifts. The new restraints account for the rise in total energy in round 22, and energy returns to the optimized level after subsequent rounds.

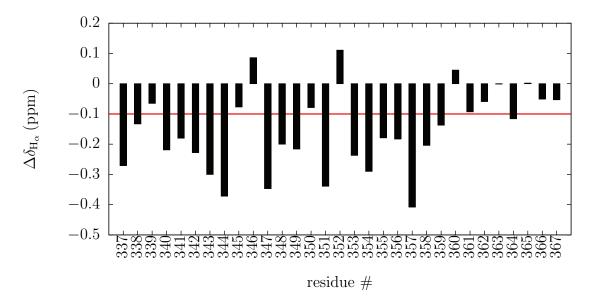


Figure 2.5: The secondary chemical shift $(\Delta\delta)$ for NHE1 TM IX H_{α} nuclei is calculated as $\delta_{H_{\alpha}} - \delta_{\rm random\ coil}$. The random coil values for each of the amino acids were obtained from (49), and observed shifts upfield to random coil values are consistent with helicity. Specifically, the widely-used chemical shift index (CSI) uses a secondary chemical shift requirement of < -0.1 as an indicator of α -helical secondary structure (50), and the red horizontal line marks this threshold.

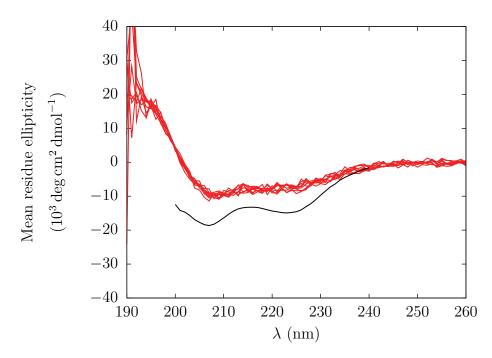


Figure 2.6: The synthetic NHE1 TM IX peptide NMR sample was diluted to produce a solution composed of ~10 μ M peptide and ~3 mM deuterated DPC (pH ~4.8). Nine replicate CD measurements were collected over three separate days on a Jasco J-810 spectropolarimeter at 30 °C in a 0.1 cm path length water-jacketed cell, and these results are plotted in red. For comparison, the predicted CD spectrum for a peptide with ~39 % α -helical content is shown in black (prediction by the K2D algorithm (23)).

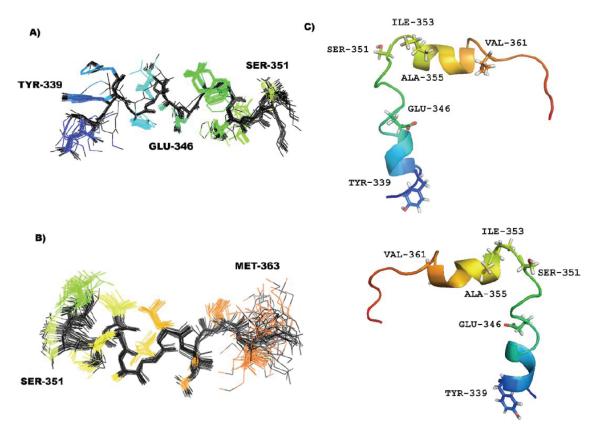


Figure 2.7: Assessment of superposable segments of the NHE1 TM IX NMR ensemble was performed using the LSQKAB program of the CCP4 suite (32), with evaluation of optimal superposition using criteria that include minimization of both local and global rmsd. The results include two superposable segments: between residues 337-350 (A) and 352-362 (B). Both of these segments have been extended in this figure to include the putative pivot point at S351, which was also found to be critical for NHE1 activity. Other functionally relevant residues (Y339, E346) and the strikingly mobile M363 (see Figure 2.8 on page 23) are also highlighted. Two alternative views of the lowest energy NHE1 TM IX NMR ensemble member are also shown (C) with colouring from N-terminus (blue) to C-terminus (red). This is a representative structure, but the angle of the kink near S351 and side chain positions are variable in the ensemble.

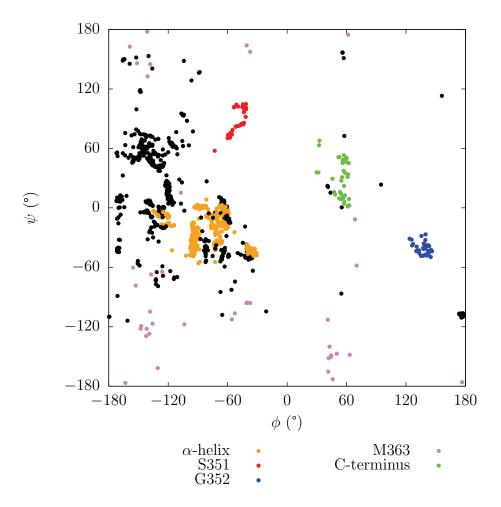


Figure 2.8: Ramachandran plot for the residues of NHE1 TM IX with ϕ and ψ values from all 40 retained structures in the final NMR ensemble. α -helical residues, the penultimate C-terminal residue, the pivot point at S351, the well-clustered G352 dihedral angles, and the strikingly variable M363 dihedral angles are all highlighted.

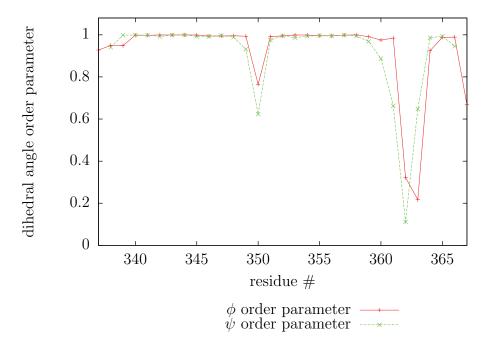


Figure 2.9: The backbone dihedral angle order parameters (ϕ, ψ) were calculated for each residue in the NHE1 TM IX construct based on the final ensemble of retained structures, as described in (51). An order parameter of 1.0 indicates a dihedral angle that is consistent over the entire ensemble of NMR structures, a value of 0.0 is consistent with a completely random distribution of dihedral angles, and intermediate values represent varied degrees of dihedral angle fluctation within the NMR ensemble. Angular standard deviation is not used because truly random angle distributions are not defined.

Table 2.1: Summary of statistics for the final retained ensemble of 40 NMR structures (out of 100 calculated) for NHE1 TM IX.

	dihedral restraints	dihedral restraints no dihedral restraints
Unique NOE restraints		
Total	1231	
Intra-residue	432	
Sequential	536	
Medium range $ i-j \le 4$	238	
Long range $ i-j > 4$	2	
Ambiguous	23	
Ramachandran plot statistics		
Core	$54.3\ \%$	
Allowed	34.6~%	
Generously allowed	7.0 %	
Disallowed	4.0~%	
XPLOR-NIH energies $(kcal/mol)^a$		
Total	54.2 ± 5.6	44.8 ± 3.1
NOE	9.95 ± 2.4	8.69 ± 2.3
NOE violations		
Violations $> 0.5 \text{ Å}$	0	0
Violations of 0.3-0.5 $\mbox{\normalfont\AA}$	13	15
Violations of 0.2-0.3 $\mbox{\AA}$	25	33

 a average deviations shown

Table 2.2: Summary of NHE1 TM IX peptide secondary structure estimates based on various algorithms available via the and submitted as input. The % structural contributions are aggregate values even for algorithms which estimate the number of DICHROWEB interface (24, 25). Nine replicates of CD data collected over three days between 260 and 190 nm were averaged discrete continguous segments for a given secondary structure type (52), and average deviations are shown with average values.

Algorithm	lgorithm Reference database	Helix (%)	Sheet $(\%)$	$\mathrm{Turn}~(\%)$	Random (%)	Total $(\%)$	Normalized rmsd
K2D	None^a	30	14	N/A	55	66	0.167
CDSSTR	4	21	34	20	25	100	0.018
CDSSTR		22	38	20	19	66	0.016
CDSSTR	SP175 (53)	19	39	∞	33	66	0.021
CONTIN-LL		31.2	29.9	15.0	23.9	100.0	0.196
CONTIN-LL	7	31.7	31.5	16.9	20.0	100.1	0.196
Average		26 ± 5	31 ± 6	16 ± 4	29 ± 10	102	

 a K2D is a neural network algorithm that does not directly require a reference protein database.

Chapter 3

NMR Spin Relaxation Studies And The Dynamics Of NHE1 TM VII (Based On The Published Manuscripts (2) And (3))

3.1 Introduction

The previous chapter (chapter 2 on page 3) introduced the biological significance of the human NHE1 protein and I presented my structural NMR studies of the ninth TM segment of NHE1. The structures of a number of other NHE1 TM segments have also been studied by NMR spectroscopy in membrane-mimetic environments as part of a "divide and conquer" approach to the study of NHE1 structure. A common theme in many of these structures is the presence of a disruption in helicitiy—a kink in the regular secondary structure which may confer structural flexibility. However, apparent flexibility in an NMR ensemble may result from a lack of observable NOE restraints and not necessarily true motion in the peptide. NMR spin relaxation experiments are well suited to probing the ps-ns and μ s-ms time scale dynamics of amino acids, and I employed ¹⁵N backbone relaxation NMR experiments to probe the dynamics at selectively labelled positions in the NHE1 TM VII peptide construct reconstituted in DPC micelles. This introduction is based on a review of NMR spin

relaxation I published (3), and followed by a section based on the NHE1 TM VII spin relaxation studies I published (2).

3.1.1 Introduction To NMR Spin Relaxation

NMR spectroscopy is a powerful technique for studying the dynamics of biomolecules using spin relaxation experiments. Frequently, when studying a protein or peptide, the objective of a backbone ¹⁵N NMR dynamics study is to identify and differentiate restricted versus mobile residues in the primary sequence and relate this information to structure and function. This type of information has lead to many biologically relevant insights, with some recent examples following. ¹⁵N relaxation analysis of PSE-4 β -lactamase revealed μ s-ms time scale mobility for residues in the active site, consistent with the ability of this enzyme to degrade a number of diverse β -lactam antibiotics (54). General odorant-binding proteins (GOBPs) also have promiscuous interaction profiles, and a recent relaxation study of honeybee GOBP ASP2 revealed increased backbone ¹⁵N-¹H ms time scale mobility at the ligand entry site (55). ¹⁵N spin-lattice (T_1) , spin-spin (T_2) , and heteronuclear nuclear Overhauser effect (NOE) relaxation measurements revealed positions of active site flexibility in the essential dihydrofolate reductase enzyme from Bacillus anthracis (56), the highly resilient causative agent of anthrax. This structure-activity relationship is important for the design of potent inhibitors.

Binding events are also frequently detected by NMR relaxation. The acidic residues in calcium-binding domain 1 of the Na⁺/Ca²⁺ exchanger are significantly restricted upon binding of calcium (57). In contrast, *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase exhibits increased ¹⁵N-¹H mobility when binding substrate analogues, mobilizing loops that may stabilize the transition state (58). Based on a September 2009 ISI Web of Knowledge (Thomson Reuters Inc.) search, nearly 2000 original research articles and 170 reviews cite the original Lipari and Szabo (6) model-free approach to NMR relaxation analysis. This demon-

strates not only the popularity of the Lipari-Szabo model-free approach but, more generally, the widespread degree to which biomolecular NMR relaxation methods are used. Accordingly, the field of NMR spin relaxation has been extensively reviewed (59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75).

Understandably, in the more than 60 years since the first detection of an NMR signal (76, 77), the methodologies and computer software employed for extracting dynamics information have diverged. In practice, several programs may be compared in the calculation of relaxation parameters. d'Auvergne and Gooley (78) present a detailed analysis of the limitations of various algorithms used for the interpretation of NMR relaxation data. For the NMR spectroscopist uninitiated to spin relaxation theory, however, the ready analysis of experimental data is compounded by the diversity of analytical approaches, the use of CGS versus SI units in defining constants and equations by different authors, and the lack of available sample calculations. It is therefore difficult to assess the quality of newly collected relaxation data without prior experience in the field. I will therefore review relaxation parameters and describe a web interface for graphing the theoretical trends of relaxation parameters to provide a first-check as to whether collected relaxation data matches with the predictions of theory. This is a crucial concern for the NMR spectroscopist preparing to embark on an exhaustive relaxation analysis for the first time. Thorough theoretical reviews of NMR spin relaxation analysis are available (i.e., (71, 69)), but are not necessarily accessible to the spectroscopist new to relaxation. Although our focus is on solution-state peptide and protein ¹⁵N-backbone NMR relaxation experiments, the fundamental principles are applicable to other biomolecular systems, and a diverse set of examples are considered that may be placed in an experimental context using the new web interface I introduce.

Before diving into the specifics, it is important to understand the microscopic origins of spin relaxation in solution-state NMR. Perhaps the most intuitive way to

understand the NMR spin relaxation process is to first consider the fully relaxed thermal equilibrium state (79). At thermal equilibrium, two important conditions are met: 1) the populations of each spin state (i.e., α and β , for a spin 1/2 nucleus) are given by the Boltzmann distribution; and 2) all coherence between α and β spin-states is lost. Spin excitation with an on-resonance radiofrequency pulse may disturb the equilibrium and break either of these conditions. For example, a π -pulse inverts the spin state populations (i.e., α and β populations are exchanged, for a spin 1/2 nucleus), breaking the condition of a Boltzmann distribution. Alternatively, a π /2-pulse produces coherent transverse magnetization, breaking the second equilibrium condition. Return to thermal equilibrium occurs by two relaxation mechanisms.

The restoration of the Boltzmann distribution, or spin-lattice relaxation (characterized by a time constant T_1), occurs as a result of the coupling of excited spins with the surrounding environment, termed the lattice for historical reasons (80). Random reorientation of molecules in a liquid modulates the local magnetic field experienced by a given spin. In typical macromolecular cases, two orientationally dependent components come into play: the magnetic fields induced by direct dipole-dipole coupling with neighbouring spins, which depend upon relative dipole orientation; and the fields caused by molecular electron currents, which depend upon molecular orientation relative to the static magnetic field. These fluctuations drive spin-lattice relaxation by inducing transitions between spin states, specifically by field fluctuations at the Larmor frequency of the nucleus.

Spin-spin relaxation (characterized by T_2) refers to the restoration of the second condition of thermal equilibrium, a loss of coherence between spins, as a result of individual variation in Larmor frequency for each spin due to local field fluctuations (81). Unlike spin-lattice relaxation, spin-spin relaxation of a given nuclear spin does not rely on a transition of *total* spin state; however, field fluctuations are dependent on spin state transitions by neighbouring nuclei.

Enhancement of magnetization relative to the thermal equilibrium level by the heteronuclear NOE is also frequently exploited for dynamics measurements in biomolecular NMR. The NOE is, of course, dependent upon the dipole-dipole distance and the relative gyromagnetic ratios of each spin (79). Assuming that these factors are known for a given pair of heteronuclei in sufficient proximity for the NOE to occur, the enhancement of longitudinal magnetization for a non-irradiated spin that is dipole-dipole coupled to a spin being irradiated by a weak radiofrequency field depends on cross-relaxation between the irradiated spin and and non-irradiated spin. The cross-relaxation rates (zero-quantum and double-quantum transitions) depend on the molecular tumbling rate (81).

It is intuitive that spin relaxation is sensitive to dynamics, the motion of bond vectors for example, because each of these relaxation processes is driven by motion. After collection of T_1 , T_2 , and NOE relaxation data, there are two main routes for mathematical analysis: 1) the Lipari and Szabo (6) model-free approach mentioned above; and 2) reduced spectral density mapping (8, 9).

The model-free approach provides intuitive motional parameters. S^2 , the order parameter, is a measure of motion amplitude and ranges from 0 for an isotropic bond vector to 1 for complete restriction of motion. S^2 does not provide sufficient information to obtain the path or model of motion for a bond vector—hence the model-free label for this technique (59). The model-free approach assumes separability of internal bond vector motion and overall molecular motion. The former timescale is quantified as the effective internal correlation time, τ_e , whereas the latter is referred to as the overall rotational correlation time, τ_c . These three model-free parameters are generally easier to interpret than the spectral density values obtained by reduced spectral density mapping.

The spectral density function, $J(\omega)$, is the Fourier transform of the rotational correlation function. In ¹⁵N biomolecular spin relaxation, the correlation function is

typically defined for the motion of a 15 N- 1 H bond vector (6). $J(\omega)$ is thus defined as the probability of observing rotational motion at a particular frequency, ω . The case of J(0), for example, could represent the (relative) population of a particular backbone 15 N- 1 H bond vector that has zero frequency, or that is rotationally immmobile, at any given time. As a whole, spectral density mapping provides the capability to demonstrate the relative degree of high- to low-frequency oscillations for a given bond vector (extensively reviwed in (64)). It has one significant advantage over the model-free approach, in that it does not require that internal bond vector motions occur independent of overall molecular tumbling.

Motional separability tends to be a particularly poor assumption for non-globular, unfolded proteins (59). As an example, Eliezer et al. (82) characterized the progression of myoglobin from an unfolded precursor to the fully folded globular protein by NMR relaxation experiments, demonstrating much greater mobility for residues in the unfolded versus folded state of the protein. Clearly, local reptation implied by dramatically increased mobility on a per-residue basis in an unfolded protein makes the separability of internal and overall motions unlikely. In any situation where this separability is suspect, spectral density mapping as opposed to, or at least in parallel to, model-free analysis may be essential (59).

Although I focus here on 15 N as a probe of local dynamics, 2 H and 13 C isotopes may also be used to study the dynamics of biomolecules, as is especially popular for the study of amino acid side chain motion. Isotope enrichment is typically employed, but it should be noted that 13 C relaxation experiments may also be performed at natural abundance (83). Uniform labelling with 13 C and fractional labelling with 2 H was used with 2 H-NMR relaxation experiments to study the side chain methyl group dynamics in the C-terminal SH2 domain of phospholipase- $C_{\gamma 1}$ (84), the first solution-state NMR study of this kind. The relaxation of a deuteron is dominated by the quadrupolar interaction that can simplify interpretation of relaxation data relative

to other nuclei, which can have multiple significant contributions to relaxation. Tugarinov et al. (85) introduced a new set of deuterium NMR relaxation experiments, allowing the study of side chain dynamics for large (~100 kDa) proteins using malate synthase G (a 723 residue single polypeptide chain enzyme) as the test system for the experiments. Experiments using different methyl isotopomer probes (CHD₂; CH₂D) were consistent with each other, with previous experiments, and with molecular dynamics simulations. A comparison of the strengths and weaknesses of ²H and ¹³C spin relaxation experiments has been conducted on recombinant human ubiquitin (86). ²H relaxation methods were better for providing methyl symmetry axis information with limited data, whereas ¹³C relaxation provided more robust model-free parameters for the time scale of methyl rotation and methyl symmetry axis motion.

3.1.1.1 Assessing The Trend Of T_1 , T_2 , And NOE With Magnetic Field Strength

Any biological insights from NMR relaxation result from translating raw experimental peak height or volume data into a set of intuitive relaxation parameters relating a residue to its mobility. The NMR pulse sequences and the calculation of initial 15 N T_1 , T_2 , and NOE values are well described by the seminal work of Farrow et al. (87). Since it is quite common to perform these experiments at multiple magnetic field strengths (8, 88), one of the first checkpoints of data integrity is the trend of T_1 , T_2 , and NOE with magnetic field strength. For this reason, I have created an online plotting tool (available at http://structbio.biochem.dal.ca/jrainey/Tyler_relaxation/) using the Python code in appendix C on page 253, which accepts a number of NMR relaxation parameters that can be tailored to a specific system to produce the theoretical trends of the relaxation times T_1 and T_2 (as well as the related rates R_1 and R_2) and the heteronuclear NOE with magnetic field strength. Initially, the user may not have an accurate estimate of certain relaxation parameters, τ_c , S^2 , τ_c , which are discussed in detail below. In these cases, the user may

input minimum and maximum values, which are plotted as a series of graded values. A set of detailed tables of parameter values for various peptide/protein systems are available in the published manuscript (3).

3.1.1.2 The Overall Rotational Correlation Time, τ_c

The overall rotational correlation time represents the time required for the molecule to tumble through one radian in an arbitrary direction (81). It depends on the size and shape of the molecule and the solvent viscosity. Some studies are consistent with the general rule of 0.5 ns overall correlation time per 1 kDa of molecular weight in a system (89, 90, 91). In contrast, there are also several cases where this estimate is not accurate (92, 93, 94). τ_c is normally observed to decrease with increasing temperature. Recombinant human ubiquitin τ_c varied as follows: 8.84 ns (5 °C), 6.36 ns (15 °C), 4.71 ns (25 °C), 3.58 ns (35 °C), 2.77 ns (45 °C), and 2.17 ns (55 °C) (95). A progressive drop in τ_c with increasing temperature was also observed for a calmodulin-peptide complex: 11.81 ns (22 °C), 8.26 ns (35 °C), 6.33 ns (47 °C), 5.00 ns (60 °C), and 4.10 ns (73 °C) (96).

In the case of proteins solvated by micelles or bicelles in solution, the "aggregate weight" versus molecular weight must be taken into account, making estimation of τ_c more difficult. For example, Yan et al. (94) proposed that a concentrated protein solution can increase the aggregation number of micellar systems, as discussed previously (97). Furthermore, 100 mmol/L NaCl can increase the SDS aggregation number from approximately 60 to 91 (98). Elevated aggregation numbers in these situations may account for increases in observed correlation times.

One simple theoretical approach for the estimation of τ_c is the Stokes-Einstein-Debye relation, which depends on isotropic motion for approximately spherical globular proteins (80). Of course, many proteins are not spherical and, even in the absence of micelles or bicelles, there is still the possibility that the protein itself aggregates in solution. More sophisticated approaches to estimation of τ_c include a relationship

between rotational correlation time and solvent accessible surface area (99), hydrodynamic calculations (100), and NMR relaxation interference experiments (101). Independent experimental measurement of τ_c may also be performed by time-resolved fluorescence spectroscopy or light scattering (80). However, most conveniently, NMR relaxation experiments are an excellent method for estimating τ_c if the protein size is less than 30 kDa (101). The normal procedure is to use a ratio of transverse $(R_2 = \frac{1}{T_2})$ and longitudinal $(R_1 = \frac{1}{T_1})$ relaxation rates (91, 102, 103, 104). Large deviations between theoretical predictions for τ_c and the experimentally determined value may suggest oligomerization.

3.1.1.3 The Generalized Order Parameter, S^2

The generalized order parameter (S^2) , introduced in the development of the model-free formalism by Lipari and Szabo (6, 7), is an intuitive way to characterize the amplitude of internal picosecond-nanosecond timescale motions of bond vectors studied by NMR relaxation experiments. S^2 values are always between 0 (fully isotropic) and 1 (a completely restricted bond vector). In the case of the extended Lipari-Szabo formalism (105), $S^2 = S_f^2 S_s^2$, where S_f^2 and S_s^2 are order parameters representing fast (ps timescale) and slow (ns timescale) internal motions, respectively. In both formalisms, the degree of motional restriction does not specify a particular model of motion, hence the "model-free" terminology.

Intuitively, S^2 is expected to decrease at higher temperature due to increased thermal motion, although only a 0.03 average decrease in S^2 was observed between 12 and 37 °C for Ribonuclease H (106). ¹⁵N-¹H NMR relaxation experiments on recombinant human ubiquitin at temperatures ranging from 5 to 55 °C also demonstrated a small drop in the order parameter as temperature was increased, with average $\frac{dS^2}{dT} = -2.3 \pm 0.95 \times 10^{-3} \text{ K}^{-1}$ (95). Similar studies on a calmodulin-peptide complex over the range of 22-73 °C also demonstrated a small $\frac{dS^2}{dT}$ of $-1.5 \times 10^{-3} \text{ K}^{-1}$, on average, with S^2 actually increasing slightly from the minimum value at 60 °C to

the value obtained at 73 °C (96). The loop residues not involved with Ca²⁺ binding had order parameters lower by almost 0.10, and had the greatest temperature dependence; notably residues 115 and 116 had $\frac{dS^2}{dT} \sim -5 \times 10^{-3}$ K⁻¹. Futhermore, ¹⁵N-H order parameters showed significantly larger changes with temperature for the unfolded states of staphylococcal nuclease (SNase) and the N-terminal SH3 domain of drk (drkN SH3) versus their respective folded states (107). For SNase, between 15 and 32 °C, $\Delta S_{avg}^2 = 0.045 \pm 0.031$, but $\Delta S_{avg}^2 = 0.143 \pm 0.032$ for the partially unfolded SNase mutant $\Delta 131\Delta$. The authors also observed this striking contrast between the folded drkN SH3 ($\Delta S_{avg}^2 = 0.004 \pm 0.036$) and the protein denatured in 2 mol/L GuHCl ($\Delta S_{avg}^2 = 0.087 \pm 0.025$) for a temperature change between 14 and 30 °C. These changes were related to the different contributions of motion to the overall heat capacity of folded and unfolded proteins. Clearly, the folding state of a protein is relevant to the temperature sensitivity of ¹⁵N-H order parameters.

An extensive database analysis of the relationship between generalized order parameter values and protein secondary structure has been conducted (108). Of the 1855 order parameters surveyed from 20 proteins, $S_{avg}^2 = 0.839 \pm 0.106$. The authors find that the backbone mobility of an amino acid is strongly correlated with its side chain size, and to a lesser extent, the size of the neighbouring residue side chains. The smallest residue, glycine, is also the most mobile $(S_{avg}^2 = 0.81 \pm 0.14)$, whereas tryptophan, which has the largest volume, is the most restricted $(S_{avg}^2 = 0.87 \pm 0.07)$. It is commonly reported that S^2 is larger in canonical secondary structure elements versus disordered loops (109), but (108) report only a slightly more mobile average for residues in loops $(S_{avg}^2 = 0.81 \pm 0.11)$ versus helices $(S_{avg}^2 = 0.88 \pm 0.07)$ or β -structures $(S_{avg}^2 = 0.85 \pm 0.07)$. Terminal residues were mobile $(S_{avg}^2 = 0.61 \pm 0.24)$, as might be expected for these often less-structured regions.

It must be noted that the 20 proteins in the database used in (108) are all stable and folded. Intrinsically disordered proteins (IDPs) are increasingly recognized as

biologically important effectors with a high binding plasticity (110, 111), which may exhibit a very different set of dynamic behaviours. Human securin, a regulator of cell division, is a 202 residue protein with 24 prolines, and is considered an IDP (112). NMR relaxation analysis is consistent with transient structuring in certain segments of securin. There is a central plateau for relaxation rates in the primary sequence and higher rates at the termini, although no model-free parameters were provided. Transient helical structuring was also observed in the IDP Sml1 based on chemical shifts, and NMR relaxation results $(R_1, R_2, \text{ and steady-state NOE})$ consistent with restricted motion for residue segments 4-20 and 60-86 (113). In another study, the Sml1 backbone structure was incredibly flexible with all $S^2 < 0.6$, except for the transiently helical regions (114). A remarkably similar degree of flexibility was observed for the backbone of the N-terminal half of hepatitis C virus core protein (C82), also an IDP, with $S_{avg}^2 = 0.59 \pm 0.04$ (115). Similarly, the natively unfolded propertide subtilisin (PPS) was flexible with $S_{avg}^2 = 0.57 \pm 0.06$ (116). To fit the PPS $^{15}{\rm N}$ relaxation data, the authors modified the traditional model-free approach by fitting each residue with a distribution of rotational correlation times to reflect the ensemble of states for the unfolded protein. S^2 and τ_e , the next parameter in our survey, were both separately fit for each individual residue. Clearly, different analytical strategies are being explored for the dynamics of natively unfolded proteins, and S^2 values are frequently lower on average than those considered canonical for structural proteins.

It has recently been suggested that the order parameters determined from modelfree analysis are merely starting values (117). A thorough assessment of N-H bond vector reorientational motion involves a variety of order parameter simulation methods that provide separate pieces of dynamic information: 1) the isotropic reorientational eigenmode dynamics method (iRED) for assessing separability of internal and overall motion as assumed by the Lipari-Szabo approach; 2) a first-order expansion in local variances and covariances accounts for contributions from local dihedral angle fluctuations to the order parameter; 3) the three-dimensional Gaussian axial fluctuation (GAF) method describes anisotropic peptide plane motion (118); and 4) the local contact model provides direct estimation of order parameter from 3D structure. Surprisingly, the local contact model produces an excellent agreement between observed S^2 values and values predicted directly from X-ray crystallography or NMR structures of lysozyme, ubiquitin, interleukin-4, calmodulin, and an HIV-1 proteaseligand complex, suggesting a strong link between structure and dynamic information (119).

3.1.1.4 The Effective Internal Correlation Time, τ_e

The effective internal correlation time (τ_e) measures the time scale for internal motions of bond vectors sweeping through an amplitude quantified by the order parameter. τ_e is notoriously difficult to interpret quantitatively because it is a complex combination of geometric factors (6, 7, 120). Low τ_e precision from model-free analysis is especially pronounced for restricted residues $(S^2 \geq 0.8)$ (121), as I have recently observed for a ¹⁵N NMR relaxation analysis of the seventh TM segment of the Na⁺/H⁺ exchanger isoform 1 (2). Palmer (68) specifically indicates that τ_e is imprecisely determined and usually not analyzed in detail. In the case of the extended Lipari-Szabo formalism, the parameter is expanded into τ_f and τ_s , which are internal correlation times for bond vector motions on picosecond and nanosecond timescales, respectively (105). The authors report ranges of 200-300 ps for τ_f and 1-3 ns for τ_s for relaxation studies on staphylococcal nuclease and interleukin-1 β . The extended method is normally used when timescales for internal motions differ by at least one order of magnitude. Typically, τ_e is on the picosecond timescale and overall tumbling occurs on the nanosecond timescale.

3.2 NMR Spin Relaxation Studies Of NHE1 TM VII

The above description of NMR spin relaxation, based on my published manuscript (3), provides the necessary foundation to understand the model-free and reduced spectral density mapping analyses of NHE1 TM VII in DPC micelles. The TM VII work is based on another published manuscript (2) and it is detailed below.

3.2.1 NHE1 TM VII Background

I have used ¹⁵N NMR relaxation methods to study the dynamics of a ¹⁵N-labelled peptide of TM VII of NHE1 and I correlate these studies with structural information. Previous studies on functional aspects of the full-length NHE1 protein were performed using alanine scanning and insertion mutagenesis at the TM VII segment (residues 251-273) (22). Ala is the fourth most common amino acid in protein TM segments and is the fifth most effective helix-inducer in a hydrophobic environment (122, 123). Ala substitutions at 13 of 22 TM VII residues resulted in severely reduced activity in the full-length NHE1 protein (22). Beyond perturbing intramolecular interactions or removing key chemical moieties required for ion transport, if flexibility at TM VII is important for NHE1 function (i.e., ion transport) Ala substitutions may interfere by promoting the structural rigidity of an α -helix in addition to replacing important charged or steric residues. The potential importance of flexibility in TM VII is spurred by the ensemble of NMR structures for the peptide in DPC micelles, which show an α -helix interrupted at G261-S263 (22). E262 is critical to activity in the full-length NHE1 protein and, more specifically, an acidic residue at this position is hypothesized to be important for cation coordination (124). Since E262D retains much of the NHE1 activity (124), it is possible that the reduced helix forming propensity of acidic residues in comparison to Ala in addition to charge retention is important for conserving both a disruption in helicity and ion coordination (22). The

G261A and E262A mutants of NHE1 were 50 % and 48 % less active than the wildtype protein, respectively, even after correction for reduced expression and targeting (22). The G261-S263 region in the TM VII peptide in DPC micelles was found in two predominant conformations, one in which G261-S263 is fairly extended and the N- and C-terminal helical regions are distal to each other and the other in which the helical regions are in close proximity and G261-S263 is allowing the formation of a tight kink. Observation of a single set of NMR chemical shifts in this region implies relatively rapid interconversion between conformations, and hence a rather dynamic structure for the TM segment despite its reconstitution in DPC micelles. Given the reduction in NHE1 activity following Ala substitution mutagenesis at G261 and E262 that were observed previously (22), a new set of mutations were also explored by our collaborators (lab of Dr. Fliegel, University of Alberta) with the goal of testing the effect of further restriction of motion in the TM VII segment. For this purpose, mutation to Ile was chosen since Ile is the most common amino acid found in protein TM segments and, of the 20 common amino acids, has the highest propensity for α -helix formation in a hydrophobic environment (122, 123). Herein, my NMR spin relaxation studies are presented in detail, while the details of collaborative mutagenesis studies are presented in the published manuscript (2).

3.2.2 Materials And Methods

3.2.2.1 Materials

Deuterium oxide (99.9% D), deuterium oxide (99.9% D) with 1% sodium 2,2-dimethyl-2-silapentane-5-sulphonate, and DPC-d₃₈ (99.1% D) were purchased from CDN Isotopes (Pointe-Claire, QC, Canada). A 535-PP NMR tube (Wilmad Glass Co., Buena, NJ, USA) was used for all NMR relaxation experiments.

3.2.2.2 Peptide Synthesis And Purification

The TM VII peptide (HINELLHILVFGESLLNDAVTVVLYKK; free N-terminus,

amide-capped C-terminus; the bold red-coloured residues having backbone ¹⁵N labels) was synthesized using solid-phase Boc chemistry (125) and purified as previously described (22). Peptide identity was confirmed by matrix-assisted laser desorption ionization mass spectrometry and by amino acid analysis (Institute for Biomolecular Design, Edmonton, AB, Canada).

3.2.2.3 NMR Spectroscopy

The NMR sample was prepared by dissolving \sim 770 μ M TM VII peptide in 90% $\rm H_2O$, 10% $\rm D_2O$ solution containing \sim 75 mM DPC-d₃₈. Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid at 1.0 mM. Solution pH was adjusted to 4.8 (deuterium isotope effects not taken into account), and all experiments were carried out at 30 °C for consistency with structural studies (22). One-dimensional 1 H observed, 15 N NMR relaxation experiments were performed on 500, 600, and 800 MHz (800 equipped with cyrogenic probe) Varian Inc. (Palo Alto, CA, USA) INOVA spectrometers, with parameters listed in Table 3.1 on page 55. The BioPack (Varian Inc.) gNhsqc pulse sequence (126) was used for measurement of 15 N relaxation of labelled TM VII residues. The 15 N relaxation rates were measured from 1D { 1 H- 15 N}-HSQC spectra. All spectra were processed and analyzed with VnmrJ 2.1B (Varian Inc.).

3.2.2.4 ¹⁵N Relaxation Parameters

¹⁵N relaxation time constants (T_1, T_2) and their standard errors from the covariance matrix were calculated using a nonlinear least-squares fit to a two parameter monoexponential decay using xcrvfit version 4.0.12¹. Errors for the first order rate constants (R_1, R_2) were propagated (127) from the time constant errors using:

$$\delta R_i = \frac{|R_i|}{|T_i|} \delta T_i \tag{3.1}$$

¹http://www.bionmr.ualberta.ca/bds/software/xcrvfit/

where R_i and T_i represent a pair of rate and time constants, respectively.

Steady-state $\{^{1}H\}^{-15}N$ NOE values were calculated using the software relax version 1.3.2 (78, 128) as the peak height (I) ratios in proton saturated versus reference spectra:

$$NOE = \frac{I_{\text{sat}}}{I_{\text{ref}}} \tag{3.2}$$

Standard deviation (σ) was propagated from the root-mean-square baseline noise as previously reported (87):

$$\sigma_{\text{NOE}} = \text{NOE} \quad \sqrt{\left(\frac{\sigma_{I_{\text{sat}}}}{I_{\text{sat}}}\right)^2 + \left(\frac{\sigma_{I_{\text{ref}}}}{I_{\text{ref}}}\right)^2}$$
 (3.3)

3.2.2.5 Model-Free Calculations

The calculated T_1 , T_2 , and NOE values at three field strengths were used to determine the model-free parameters τ_M (overall rotational correlation time), S^2 (generalized order parameter), and τ_e (effective internal correlation time) through spectral density function fitting using the following relaxation expressions (129, 8):

$$\frac{1}{T_1} = \left(\frac{d^2}{4}\right) \left[J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) \right] + c^2 J(\omega_N)$$
 (3.4)

$$\frac{1}{T_2} = \left(\frac{d^2}{8}\right) \left[4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)\right] + \left(\frac{c^2}{6}\right) \left[3J(\omega_N) + 4J(0)\right]$$
(3.5)

NOE = 1 +
$$\left(\frac{d^2}{4}\right) \left(\frac{\gamma_H}{\gamma_N}\right) [6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)]T_1$$
 (3.6)

where $d = \left[\frac{\mu_0 h \gamma_N \gamma_H}{(8\pi^2)} \left\langle \frac{1}{r_{\rm NH}^3} \right\rangle \right]$, $c = \left(\frac{\omega_N}{\sqrt{3}}\right) (\sigma_{\parallel} - \sigma_{\perp})$, μ_0 is the permeability of free space, ω_N and ω_H are the respective nuclear Larmor frequencies of ¹⁵N and ¹H, γ_N and γ_H are the respective gyromagnetic ratios of ¹⁵N and ¹H, h is Planck's constant, $r_{\rm NH}$ is the length of the amide bond, and σ_{\parallel} and σ_{\perp} are the parallel and perpendicular

components of the axially symmetric chemical shift tensor. A value of -160 ppm was used for $(\sigma_{\parallel} - \sigma_{\perp})$ (87, 130). The form of the spectral density function used in the Lipari-Szabo formalism is given by (6, 7):

$$J(\omega) = \frac{2}{5} \left\{ \frac{S^2 \tau_M}{[1 + (\omega^2 \tau_M^2)]} + \frac{(1 + S^2)\tau}{[1 + (\omega\tau)^2]} \right\}$$
(3.7)

where $\frac{1}{\tau} = \frac{1}{\tau_M} + \frac{1}{\tau_e}$.

Fitting of the model-free parameters τ_M , S^2 , and τ_e to the relaxation data was performed using a suite of Mathematica (Wolfram Research Inc., Champaign, IL, USA) notebooks previously described (131) but modified by Spyracopoulos to include data collected at multiple field strengths in a single calculation. Briefly, five forms of the spectral density function are considered to account for mixtures of motion on various time scales (131, 132). An optimization procedure is performed to fit the experimental input $(T_1, T_2, \text{ steady-state NOE})$ to each of these five mathematical models, labelled 1-5. The appropriate model for each of the residues is selected using the statistical approach of Akaike's information criteria (AIC) (133), and 100 Monte Carlo simulations were performed to estimate parameter errors (121).

3.2.2.6 Reduced Spectral Density Mapping

Using the reduced spectral density mapping approach (8, 9), measurement at three field strengths for the steady-state NOE, spin-lattice (T_1) and spin-spin (T_2) relaxation times of ¹⁵N allows for sampling of seven spectral density values describing the motion of the system (88). The spectral density values, under the high frequency approximation that $J(0.921\omega_H)$ and $J(0.955\omega_H)$ are both equivalent to $J(0.870\omega_H)$

(8), are obtained from the following set of equations:

$$NOE = 1 + \left(\frac{d^2}{4}\right) \left(\frac{\gamma_H}{\gamma_N}\right) \left[5J(0.870\omega_H)\right] T_1$$
(3.8)

$$R_1 = \left(\frac{d^2}{4}\right) \left[3J(\omega_N) + 7J(0.870\omega_H)\right] + c^2 J(\omega_N)$$
(3.9)

$$R_2 = \left(\frac{d^2}{8}\right) \left[4J(0) + 3J(\omega_N) + 13J(0.870\omega_H)\right] + \left(\frac{c^2}{6}\right) \left[3J(\omega_N) + 4J(0)\right]$$
(3.10)

Equations 3.8 to 3.10 allow solving for the three unknown spectral density functions $J(0.870\omega_H)$, $J(\omega_N)$, and J(0). The field-dependent $J(0.870\omega_H)$ and $J(\omega_N)$ alongside the field-independent J(0) give seven spectral density values for a given bond vector at three field strengths. Uncertainties in the values of spectral density functions were calculated by propagating the uncertainties of the independent variables using a sum of squares equation:

$$\delta q = \sqrt{\left(\frac{\delta q}{\delta x}\delta x\right)^2 + \dots + \left(\frac{\delta q}{\delta z}\delta z\right)^2}$$
 (3.11)

where x ... z represent any number of independent variables and δ values are parameter uncertainties (127).

3.2.2.7 Theoretical Calculations

The software Maple 11.02 (Waterloo Maple, Inc., Waterloo, ON, Canada) was used to predict the trend for T_1 , T_2 or NOE as a function of magnetic field strength according to equations 3.4-3.7 (prior to the development of the web-accessible program described in section 3.1.1.1 on page 33). Specifically, each of the three model-free parameters used to describe the motion of ¹⁵N-H bond vector (S^2, τ_e, τ_M) was independently varied to study the robustness of the trend of T_1 , T_2 or NOE with increasing magnetic field strength. τ_M of the NHE1 TM VII peptide in DPC micelles was esti-

mated using a T_1/T_2 fit strategy in the Mathematica notebooks introduced above, as previously described (87, 131).

3.2.3 Results

3.2.3.1 Relaxation Parameters: T_1 , T_2 , and NOE

A set of ¹⁵N NMR relaxation data ($T_1 = \frac{1}{R_1}$, $T_2 = \frac{1}{R_2}$, and NOE) was acquired at 500, 600, and 800 MHz for a specifically ¹⁵N labelled TM VII peptide in DPC micelles at 30 °C (*i.e.*, Figure 3.1 on page 52). The T_1 values are similar for all six ¹⁵N-labelled TM VII residues, with slightly lower values for L254 at 500 MHz and 600 MHz but not at 800 MHz (Figure 3.2 on page 53). There is a trend toward increasing T_1 at higher field strength, although the increase is within the bounds of experimental error. In contrast, T_2 values clearly decrease at higher magnetic field strength. For each magnetic field strength, the T_2 values are approximately constant over the six residues. The steady-state NOE values are also approximately constant over the six ¹⁵N-labelled residues at a given field strength. Although NOE values compared between different field strengths overlap within the bounds of experimental error, there is a trend toward increasing NOE at higher field. The complete set of relaxation parameter values and errors are presented in Table 3.2 on page 56.

3.2.3.2 Reduced Spectral Density Mapping

 $J(0.870\omega_H)$, calculated directly from equation 3.8, is plotted for the six ¹⁵N-labelled TM VII residues and values are compared between field strengths in Figure 3.3 on page 54. The general trend is a decrease in $J(0.870\omega_H)$ with increasing field strength, although there is overlap between values within the bounds of experimental error, and in the case of G261 $J(0.870\omega_H)$ is slightly greater at 800 MHz versus 600 MHz. From a sequential standpoint, all six ¹⁵N-labelled residues have very similar $J(0.870\omega_H)$. Using the calculated $J(0.870\omega_H)$ and experimental T_1 values, equation 3.9 can be solved for $J(\omega_N)$. $J(\omega_N)$ values calculated from 500 MHz and

600 MHz experiments overlap within the bounds of experimental error, but values calculated from 800 MHz data are clearly smaller. In terms of primary sequence, none of the six 15 N-labelled residues differs within the bounds of experimental error. Finally, experimental T_2 values and calculated $J(\omega_N)$ and $J(0.870\omega_H)$ values can be used to solve equation 3.10 for J(0). There is considerable overlap in J(0) across field strengths within the bounds of experimental error, although there is a trend toward higher values at 800 MHz. However, from a sequential standpoint, none of the six residues has a significantly different J(0) at a given field strength.

3.2.3.3 Model-Free Analysis

The most common analysis of ¹⁵N amide relaxation data for proteins and peptides is the Lipari-Szabo model-free approach where three parameters $(S^2, \tau_e, \text{ and } \tau_M)$ describe the motion of a ¹⁵N-H bond vector based on spectral density functions (i.e., equation 3.7) (6, 7). The complete model-free results are summarized in Table 3.3 on page 57. For L254, L258, and L273, AIC selection favored model five, but each fit had a highly skewed χ^2 distribution so the next most likely model was chosen. This is also preferable because model five has the most fitting parameters (four), which can increase the strength of fit independent of its true reflection of the empirical relaxation data. The AIC approach selected model four for G261, L264, and A268, which includes a chemical exchange term, $R_{\rm ex}$ —defined as a relaxation contribution from μ s-ms time scale motions (8). This is consistent with a strand of the peptide spanning from G261 to A268 that is subject to chemical exchange. G261 has a slightly lower order parameter ($S^2 = 0.65 \pm 0.02$) than the other five ¹⁵N-labelled residues $(S_{average}^2 = 0.80 \pm 0.02)$. For G261, the slightly reduced order parameter is not consistent with an elevated J(0) (Figure 3.3). Although neither J(0) or S^2 is remarkably different from those of the other five residues, it is noteworthy that chemical exchange $(R_{\rm ex})$ motions in the μ s-ms range can inflate J(0) for affected residues (88, 134):

$$J(0)_{\text{obs}} = J(0)_{\text{corr}} + \lambda R_{ex} \tag{3.12}$$

where λ is a positive scaling factor, $\lambda = (\frac{3}{2})[\frac{1}{3d^2+c^2}]$; c and d are the chemical shift and dipolar constants defined in section 3.2.2; and $J(0)_{\text{obs}}$ and $J(0)_{\text{corr}}$ are the values before and after correction for the contribution from chemical exchange, respectively. Since G261 has the largest R_{ex} value estimated from model-free analysis (1.9 ± 0.2) s⁻¹ (Table 3.3), it is certainly possible that chemical exchange can account for the discrepency of $J(0)_{\text{obs}}$ and order parameter for G261. I avoid a more detailed consideration of chemical exchange since accurate quantification requires relaxation dispersion experiments (88, 135).

3.2.4 Discussion

3.2.4.1 Relaxation Parameters: Comparing Theory And Experiment

The six 15 N-labelled residues of the TM VII peptide have similar values for all three relaxation parameters at each field strength (Figure 3.2 on page 53) suggesting similar flexibility on the ps-ns time scale along the length of the peptide. Both T_1 and T_2 follow the expected theoretical trends within experimental error for relaxation time versus field strength at the experimentally determined rotational correlation time (τ_M) of ~10 ns. Analysis of the steady-state NOE is a bit more convoluted. The predicted trend is a decrease in the NOE with increasing field strength for a τ_e in the tens of ps range as estimated from model-free analysis (Table 3.3 on page 57) coupled with the experimentally estimated τ_M . However, we generally observe that NOE increases with field strength (Figure 3.2). It is possible that the effective internal correlation times are underestimated, in which case a τ_e of 350 ps would be sufficient to account for the observed trend. This is not surprising given the low precision in the τ_e estimations from model-free analysis (Table 3.3). Low τ_e precision from model-free

analysis is especially pronounced for restricted residues ($S^2 \ge 0.8$) (121).

3.2.4.2 Reduced Spectral Density Mapping

No significant differences were observed for $J(0.870\omega_H)$, $J(\omega_N)$ or J(0) at a given field strength for the six ¹⁵N-labelled residues (Figure 3.3 on page 54), again suggesting that motions on the ps-ns time scale are similar for the tested residues. The propagated errors at the three spectral density frequencies vary considerably, and also depend on the field strength of measurement. The former result is consistent with previous NMR dynamics analyses on a series of peptides where errors varied between negligible and large on a per-residue basis at a given spectral density frequency (83). The most commonly used measure of structural flexibility in spectral density mapping is J(0), the value of the function at zero frequency. It is normally interpreted as a measure of restricted motion, with large values suggesting increased local structure, and small values consistent with flexibility (88, 83). Chemical exchange $(R_{\rm ex})$ and ¹⁵N chemical shift anisotropy (CSA) variations along the primary sequence of the peptide may contribute to errors in J(0) and may explain the deviations between field strengths we observed (Figure 3.3) for this theoretically field-independent parameter (88). Specifically, J(0) values calculated from higher field strength data are more susceptible to inflation by chemical exchange contributions to R_2 (88), and our J(0) values are generally greater using 800 MHz data (Figure 3.3). J(0) values are similar for all six ¹⁵N-labelled residues, suggesting an equal degree of motional restriction on the ps-ns time scale. The slightly reduced order parameter for G261 does not contradict its J(0) because chemical exchange can inflate the observed J(0)(88, 134). If G261 is a pivot point allowing motion of the portions of TM VII Cand N-terminal to it relative to each other, as we have previously suggested (22), the motion at this residue is most likely to be on the μ s-ms time scale. This estimate of the motional time scale is based both on the observation of a single set of exchangeaveraged chemical shifts despite extensive conformational sampling in the peptide apparent from nuclear Overhauser effect contacts (22) and on the mathematical fit of a chemical exchange term $(R_{\rm ex})$ from our model-free analysis (Table 3.3).

3.2.4.3 Correlating Structure, Dynamics, And Function

The structure of TM VII in DPC micelles is an interrupted α -helix (22). For a converged structural ensemble without discarding a significant portion (> 34%) of the NOE contacts assigned, it was necessary to employ a new dual-conformer calculation protocol (22, 29). Through parallel calculation of pairs of conformers, all NOEs were satisfied, implying extensive conformational sampling about the G261-S63 region of the TM segment. The dual-conformer protocol is equally accommodating of pairs of non-interacting conformers existing simultaneously in the ensemble vs. oligomerization. Because NOEs were almost entirely satisfied through isolated dual conformers, rather than dimer formation, TM VII was attributed to be a monomer undergoing conformational exchange (22). The TM VII peptide gave a single set of averaged chemical shifts, rather than multiple sets of independently sequentially assignable shifts implying distinct and long-lived conformations (*i.e.*, our recent structure of apelin-17 (136)). Chemical shift averaging demonstrates that interconversion between the two major conformers assumed by TM VII in micelles is rapid on the NMR time scale (~ms or faster).

Placing this in context of 15 N-backbone relaxation, the highly similar T_1 , T_2 , and steady-state NOE values imply highly similar dynamics at the ps-ns time scale along the length of the TM VII peptide. In light of both the chemical shift averaging and the lack of distinctive variations in ps-ns dynamics, conformational interconversion is therefore most likely to be in the μ s-ms regime. To produce the observed dual-conformer set of TM VII structures, this exchange must be occurring in the G261-S263 region and is consistent with a chemical exchange model being selected from model-free analysis for residues G261, L264, and A268 (Table 3.3 on page 57), with the largest $R_{\rm ex}$ parameter for G261. From these model-free results, a hypothetical

mechanism is that the N-terminal region of TM VII is undergoing relatively little μ s-ms level motion while the G261-S63 pivot point is allowing the C-terminal region containing L264 and A268 to exchange between two conformations relative to the N-terminus.

In order to consider the necessity of flexibility allowed by the break in helicity of the otherwise helical TM VII segment at residues G261-S263, I compare two sets of mutagenesis studies performed by our collaborators (for the details of the functional studies see (2, 22)). E262 is not considered in this structural-dynamic correlation analysis, given the likelihood of involvement in ion translocation (14). There is a perturbation to function with Ala mutation and almost complete loss of function with Ile mutation for the full-length NHE1 protein with mutation at F260, G261 and S263. Liu and Deber have tested the propensity for all 20 amino acids to form an α -helix in a hydrophobic environment (122, 123). Using these results, the effect of the various Ala and Ile mutations presented both previously (22) and herein can be examined in the context of a perturbation to the formation of a non-helical region. Mutation of F260, which is in the N-terminal α -helical region of TM VII, to Ala or Ile would be fairly non-perturbing in terms of secondary structure propensity and F260I reasonably conservative in terms of side-chain size; therefore, the loss of the F260 side-chain itself appears to be the critical feature of this mutation. Both G261A and G261I are relatively non-perturbing in terms of α -helical propensity in a membrane. For this position, therefore, the lack of steric constraint of a Gly residue seems likely to be the most important factor, correlating well to the break in helical structure and to the evidence for μ s-ms scale dynamics at G261. S263 is sensitive to mutation to either Ala or Ile, with mutation to either residue significantly increasing helical propensity. A good possibility is that the Ala and Ile mutants are sufficiently perturbing to local structure to extend the helical segment beginning at L264 to include S263A or S263I. This would significantly perturb both structure and dynamics in the G261-S263

region.

Interruptions in regular secondary structure have now been documented for NHE1 TM segments IV, VII, IX, and XI (22, 137, 1, 27). It will be important to assess the theme of intermediate time scale motion about a pivot point such as that predicted herein with more detailed chemical exchange information by performing relaxation dispersion experiments (88, 135). Structural and dynamics characterization of mutant TM domains which are known to significantly perturb function are also a potentially valuable tool in terms of understanding these processes. Chemical exchange is entirely consistent with the alternating-access mechanism proposed for exposure of E262 to cytosolic protons during the ion translocation cycle of NHE1, where the residue is bent away from the cytosol in certain conformations (14). An alternating-access mechanism was also proposed for the homologous NhaA protein on the basis of its crystal structure (42).

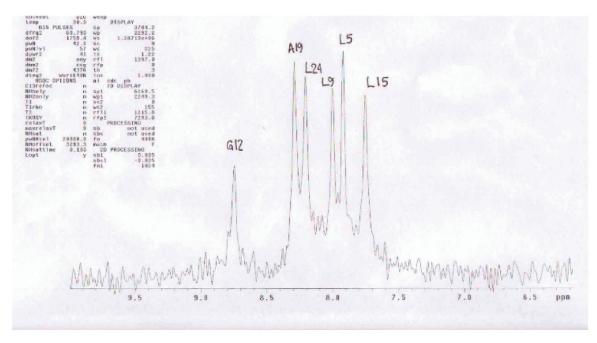


Figure 3.1: The integrity of the NHE1 TM VII sample in DPC micelles was verified by collection of a one-dimensional $^1\mathrm{H}$ - $^{15}\mathrm{N}$ NMR spectrum on a 600 MHz spectrometer (128 scans). The spectrum is consistent with results from a previous pure sample of NHE1 TM VII (not shown).

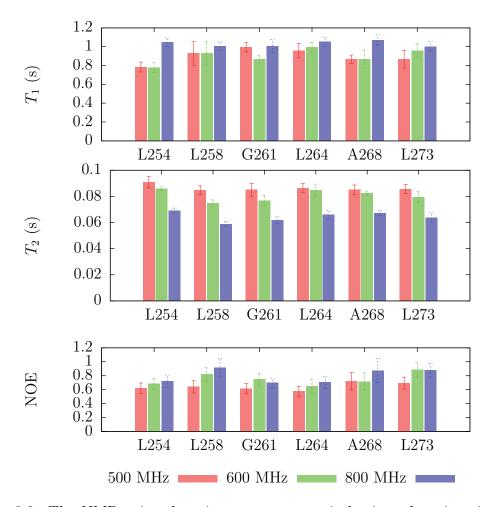


Figure 3.2: The NMR spin-relaxation parameters spin-lattice relaxation time (T_1) , spin-spin relaxation time (T_2) , and steady-state NOE are plotted for measurements at three magnetic field strengths for each of the ¹⁵N backbone-labelled positions in the NHE1 TM VII peptide construct. Each residue is numbered according to its position in full-length NHE1.

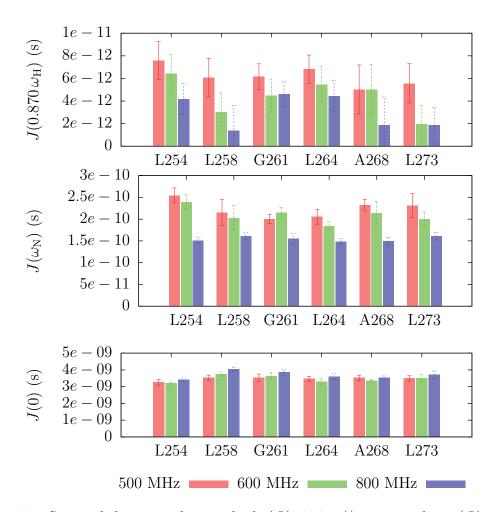


Figure 3.3: Spectral density values at high $(J(0.870\omega_{\rm H}))$, intermediate $(J(\omega_{\rm N}))$ and zero (J(0)) frequencies are shown for each of the six ¹⁵N-labelled NHE1 TM VII residues for measurements at three magnetic field strengths. The spectral densities represent the population of ¹⁵N-H bond vectors rotating at a given frequency, and the results are similar for each residue at a given field strength within the bounds of uncertainty.

Table 3.1: NMR parameters for 15 N relaxation experiments at 500, 600, and 800 MHz.

Table 3.2: The full set of longitudinal relaxation rate (R_1) , transverse relaxation rate (R_2) , and steady-state NOE ¹⁵N amide relaxation parameters for NHE1 TM VII in DPC micelles.

Besidne	Assidne B. (s^{-1}) B. (s^{-1}) B. (s^{-1})	$B, (s^{-1})$	$B_{*}(s^{-1})$	$B_{\rm s} ({\rm s}^{-1})$	$B_{\rm s} ({\rm s}^{-1})$	$B_{\rm s} ({\rm s}^{-1})$	NOE	NOE	NOE
Icarac	(6) [17	(2) 127	(6) 127	(2) 731	102 (2)	(2) 731	101		
	500 MHz	2HM 009	800 MHz	$500 \mathrm{\ MHz}$	600 MHz	800 MHz	$500 \mathrm{\ MHz}$	2HM 009	800 MHz
L254	1.28 ± 0.08	1.28 ± 0.08 1.29 ± 0.09 $0.96 \pm 0.$	0.96 ± 0.04	11.02 ± 0.52	11.65 ± 0.20	11.65 ± 0.20 14.47 ± 0.41 0.62 ± 0.08	0.62 ± 0.08	0.68 ± 0.08 0.72 ± 0.09	0.72 ± 0.09
L258	1.08 ± 0.14	1.08 ± 0.14 1.07 ± 0.15 1.00 ± 0.04	1.00 ± 0.04	11.80 ± 0.46	13.38 ± 0.46	16.99 ± 0.53	0.64 ± 0.09	0.82 ± 0.10	0.91 ± 0.14
G261	1.01 ± 0.05	1.15 ± 0.06	0.99 ± 0.07	11.76 ± 0.68	13.06 ± 0.71	16.23 ± 0.72	0.61 ± 0.07	0.75 ± 0.08	0.70 ± 0.07
L264	1.04 ± 0.08	1.00 ± 0.05	0.95 ± 0.04	11.56 ± 0.44	11.81 ± 0.66	15.16 ± 0.79	0.58 ± 0.07	0.65 ± 0.10	0.70 ± 0.09
A268	1.15 ± 0.06	1.15 ± 0.13	0.93 ± 0.05	11.77 ± 0.52	12.13 ± 0.24	14.86 ± 0.47	0.72 ± 0.12	0.72 ± 0.12	0.87 ± 0.17
L273	1.15 ± 0.13	1.05 ± 0.08 1.00 ± 0.05	1.00 ± 0.05	11.68 ± 0.49	12.58 ± 0.69	15.66 ± 0.88	0.69 ± 0.09	0.88 ± 0.10	0.88 ± 0.10

Table 3.3: Spectral density model selections and model-free parameters estimated for each of the 15 N-labelled NHE1 TM VII residues. Large uncertainties in the measurement of τ_e are well-documented (69).

Residue	Model	S^2	$\tau_e \text{ (ps)}$	R_{ex} (s ⁻¹)
L254	2	0.78 ± 0.01	40 ± 11	
L258	2	0.89 ± 0.02	37 ± 229	
G261	4	0.65 ± 0.02	16 ± 5	1.9 ± 0.2
L264	4	0.75 ± 0.02	36 ± 8	0.6 ± 0.3
A268	4	0.76 ± 0.02	24 ± 13	0.5 ± 0.2
L273	1	0.85 ± 0.02		

Chapter 4

Technicalities Of Spitz Preparation

4.1 Introduction

Spitz is a TM protein substrate of rhomboid-1 (Figure 4.1), an intramembrane serine protease localized to the Golgi apparatus in the *Drosophila* secretory pathway. Rhomboid is named for the abnormal rhomboid-shaped head skeleton observed in a Drosophila mutant (138). It was later found that rhomboid-1 cleavage of spitz is important for the release of this *Drosophila* epidermal growth factor receptor (EGFR) ligand and that proteolytic activity is controlled by Golgi compartmentalization of enzyme and controlled trafficking of substrate by another protein, Star, from the endoplasmic reticulum (ER) to Golgi (139, 140). Indeed, spitz is the principal activating ligand for Drosophila EGFR and is similar to mammalian TGF_{α} . While Star is clearly required to chaperone spitz from the ER to the Golgi, it is not involved in the actual cleavage of spitz (139). The cleavage of spitz was clearly established to occur in the Golgi and the luminal fragment is then trafficked to the plasma membrane and released for EGFR signaling. Drosophila rhomboid-1 was the first example of an intramembrane serine protease, but a number of others have since been identified across all kingdoms of life (reviewed by (10)). The sequence diversity between rhomboids in different organisms is striking, and only loose sequence requirements have been established for substrate cleavage (141). There is, however, some agreement that postulated helix-breaking residues are required in the substrate for cleavage to occur.

There are many biologically-relevant examples of rhomboid homologues. The bacterial rhomboid AarA from *Providencia stuartii* cleaves part of the twin-arginine translocase subunit, TatA, and this process is required for the release of a quorum sensing ligand (142). Although TatA oligomerizes to allow for folded bacterial proteins to exit the cell, it is not yet clear if the quorum sensing ligand is directly released by the oligomerization of TatA. Nonetheless, *P. stuartii* is an opportunistic human pathogen, and quorum sensing is involved in its antibiotic resistance (143). Futhermore, apicomplexan rhomboids can cleave cell-surface adhesins, and this appears to be important for invasion of host cells by *Plasmodium falciparum* (144), the parasite responsible for human malaria. Yeast mitochondrial rhomboids are known to regulate membrane remodelling (145). Similarly, the mitochondrial rhomboid PARL (presenilin-associated rhomboid-like) regulates remodelling of cristae and apoptosis in mice (146).

There is clearly a diverse set of rhomboid functions in different organisms, and there are likely many functions yet to be uncovered. Surprisingly, there are no rhomboid-specific inhibitors despite the fact that rhomboids are not phylogenetically related to their soluble serine protease counterparts (reviewed in (10)). Indeed, rhomboids use a Ser-His catalytic dyad unlike their soluble counterparts which require a catalytic triad involving aspartic acid (147). Furthermore, although it has been five years since the first reported rhomboid protease crystal structure, that of E. coli GlpG (4), it remains unclear how the substrate enters the active site of the protease—which is surrounded by the helices of the polytopic rhomboid. Some convincing mutagenesis work shows that GlpG TM5 is likely a substrate gate (148), consistent with the variations in TM5 position reported between several different rhomboid crystal structures (reviewed in (149)).

Most structural studies to date on the spitz-rhomboid or homologous systems have focused on the protease rather than the TM substrate. The recent crystallization of *E. coli* GlpG with an irreversible (covalent) mechanism-based isocoumarin inhibitor is a step in the right direction (150), but still does not fully address how a peptide-sized substrate can laterally enter the TM core of the enzyme. To my knowledge, there is no high-resolution structure available for a validated rhomboid substrate. With the biological incentives for understanding rhomboid proteases highlighted above, we endeavoured to produce the first high-resolution structure of a spitz TMD construct. A number of attempts at producing and purifying a variety of spitz and related TMD constructs are described in this chapter. Clearly, the peptides are not very tractable. Although it was possible to collect NMR spectra for one of the preparations (see section 4.4 on page 62), it has not been possible to solve the high-resolution NMR structure yet. Additional studies on the spitz-rhomboid system were performed using coarse-grained molecular dynamics simulations (see chapter 6 on page 191).

4.2 Solid-Phase Peptide Synthesis

My primary strategy for production of rhomboid protease substrate constructs was the use of Fmoc (9-fluorenylmethoxycarbonyl) solid-phase peptide synthesis (SPPS). Fmoc-protected amino acids, Fmoc-Lys(Boc)-Wang resin (0.57 mmol/g), Fmoc-Ile Wang resin (0.55 mmol/g), O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), and 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) were from aapptec (Louisville, KY). Fmoc-Lys(Boc)-Wang resin (0.19 mmol/g) was from Otwo Biotech (Guangdong, China). Side chain protecting groups on Fmoc amino acids were 2,2,4,6,7-pentamethyl-dihydrobenzofuran (Pbf) for Arg; triphenylmethane (Trt) for Gln and His; tert-butyl (tBu) for Ser; and tert-butoxycarbonyl (Boc) for Lys. N,N-dimethylformamide (DMF, sequencing grade) was obtained from Fisher Scientific (Ottawa, ON). N,N-

diisopropylethylamine (DIEA) was consistently used to facilitate coupling reactions while piperidine was used for Fmoc deprotection reactions. HBTU was used as the coupling reagent for most reactions, but HATU was used for coupling with more costly amino acids (especially those with isotopic labels), and for couplings that follow the incorporation of His or Cys. For problematic coupling reactions, after several rounds of attempted coupling, an acetylation reaction with acetic anhydride was normally performed to cap unreacted sites. The Kaiser (151) and isatin (152) colourimetric tests were used to monitor the completion of reactions.

4.3 Production And Purification Of A 34-Residue Spitz TMD Construct (tr-08-1)

The Fmoc solid-phase peptide synthesis of a 34-residue construct (KRPRPM-LEKASIASGAMCALVFMLFVCLAFYLRK; designated tr-08-1) of the spitz TMD was performed using 0.6 g of Fmoc-Lys(Boc)-Wang resin (0.57 mmol/g loading). After cleavage from the synthesis resin, the crude compound was largely insoluble in a number of solvents: H_2O , trifluoroacetic acid (TFA), isopropanol, acetonitrile (ACN), and dimethyl sulfoxide (DMSO). After a few days of lyophilization in a mixture of these solvents, \sim 50 mg of crude peptide was dissolved in 3 mL of 100% TFA. The TFA solution was diluted with 50% H_2O/ACN , and a \sim 0.7 mg/mL crude solution was subjected to C3 reverse-phase high performance liquid chromatography (HPLC) (not shown). Mass spectrometry results were consistent with the presence of the target product, but the HPLC profile exhibited substantial impurities.

Many HPLC runs were performed and DTT treatment seemed to improve the traces, so disulfide bond formation may have contributed to the reduced solubility and tractability of the peptide. However, there were persistent inconsistencies in peak elution times in matching runs and we decided to produce the same peptide construct with Cys residues replaced by Ser in an attempt to improve handling (section 4.4 on

page 62). In retrospect, I think it would have been worth performing several rounds of crude lyophilization in the presence of excess H₂O, as this approach often improved the solubility of subsequent synthetic spitz constructs.

4.4 Production And Purification Of A 34-Residue C→S Spitz TMD Construct (tr-08-2)

The substantial problems with peptide handling experienced working with the TR-08-1 construct (section 4.3 on page 61) may relate to the formation of disulfide bonds and oligomerization. I repeated the SPPS procedure using Ser in place of both Cys residues. The new construct, labelled TR-08-2, was cleaved from the resin to yield a yellow gel-like crude product that dissolved in 25% $\rm H_2O$, 25% ACN, 50% formic acid. Lyophilization over several days with excess water produced a white powder. Initial electrospray ionization mass spectrometry (ESI-MS) results were consistent with the presence of 3+, 4+, 5+, and 6+ charged species of the \sim 3828 g/mol peptide (not shown).

The purity of the crude sample was further assessed by C18 analytical HPLC (Figure 4.2 on page 74) and there were a number of species present. The MALDI-MS results (not shown) were consistent with the elution of product in fraction 7 (30-35 minutes). TR-08-2 samples were concentrated by optimizing the solvent—optimal reconstitution was achieved in 60% deionized water (DI-H₂O), 40% ACN. After multiple attempts at HPLC gradient optimization for purification (not shown), one of the most promising fractions produced an encouraging MALDI-MS result (Figure 4.3 on page 75). We were reasonably satisfied with the purity demonstrated by this MALDI spectrum (although in retrospect I'd be more concerned about the low molecular weight species), and decided to purify the entire ~19 mL crude stock. At the time we were limited to a 250 μ L sample loop, and I completed 76× 48-minute HPLC runs to purify the crude (see Figure 4.4 on page 76 for a representative HPLC trace).

The pooled TR-08-2 samples produced using the above workflow were lyophilized and further HPLC purification was performed (Figure 4.5 on page 77). Curiously, the MALDI-MS results for fraction 1 (Figure B.35 on page 251) and fraction 2 (Figure B.36 on page 252) similarly reflected a relatively pure sample with major species ~ 3830 g/mol, and the results were also verified by ESI-MS (not shown). Thus, both fractions were collected via many additional rounds of HPLC purification, yielding 0.8 mg and 2.4 mg of fractions 1 and 2 respectively. An NMR sample was prepared with the following composition: 1 mM DSS, 95% $\rm H_2O/5\%$ D₂O, ~ 75 mM DPC-d₃₈, $\sim 260~\mu$ M peptide (TR-08-2 fraction 1), and pH 5. A matching sample (but slightly more dilute at $\sim 100~\mu$ M fraction 1, 32 mM DPC) was used for circular dichroism spectropolarimetry (Figure 4.6 on page 78). The peptide appears to be α -helical, and qualitatively similar results were obtained for a very dilute solution of fraction 2. However, TOCSY (total correlation spectroscopy) and HSQC (heteronuclear single quantum correlation) NMR experiments on a 500 MHz spectrometer had insufficient signal, and the described sample may have been too dilute (not shown).

A more concentrated NMR sample was made with TR-08-2 fraction 2 with the following composition: 0.96 mM peptide, ~75 mM DPC-d₃₈, 95% H₂O/5% D₂O, 1 mM DSS, and pH 5. Although reasonable signal/noise could not be obtained on a 500 MHz spectrometer, high quality NMR spectra were obtained on a 700 MHz magnet (fitted with a cryogenically cooled probe). 2D NOESY (200 ms mixing time, Figure 4.7 on page 79), TOCSY (Figure 4.8 on page 79), and natural abundance ¹³C-HSQC spectra were used for resonance assignment. However, as demonstrated in the TOCSY caption, there is substantial resonance overlap and after several weeks of attempted assignment it was deemed unlikely that the homonuclear NMR data would suffice for structure determination. The next step was to produce a construct with isotope labels to facilitate resonance assignment (section 4.5 on page 64).

4.5 Production And Purification Of A 21-Residue Spitz TMD Construct (tr-09-1)

A 21-residue spitz TMD peptide construct was designed as outlined in Figure 4.9 on page 80. The Fmoc solid-phase peptide synthesis of this construct (designated tr-09-1) was performed with 0.6 g of Fmoc-Lys(Boc)-Wang resin (0.57 mmol/g) and includes the incorporation of selective fractional ¹⁵N backbone labels. Predicting the isotopic mass of the major expected product requires a 'tree-branch' analysis approach (Figure 4.10 on page 81). The latter analysis suggests that major products will incorporate 4-5 ¹⁵N backbone labels. Accounting for the natural abundance of ¹³C and the incorporation of an additional proton in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, the expected mass of the product is ~2249-2250 g/mol. I wrote an internet-accessible python program (available at: http://129.173.89.133/cgi-bin/isotope_cgi.py) for performing these fractional isotope calculations and the underlying code is included in module A.1 on page 206.

As anticipated from work with previous spitz TMD constructs, there were substantial problems with the solubility of tr-09-1. None of the following solvents could dissolve the crude peptide after cleavage from the resin: ACN/H₂O mixtures, methanol, isopropanol, chloroform, hexane, or DMSO. One possibility is that disulfide crosslinking reduces the solubility of the crude peptide, but a 70% H₂O, 30% ACN, 200 mM dithiothreitol (DTT) mixture with trace TFA could not dissolve the product, even after treatment at 80 °C. While the crude peptide was also insoluble in 50% H₂O/50% TFA, it was finally possible to dissolve the crude peptide in a 100% TFA solution. However, the lyophilization product did not have an improved appearance or consistency—it was a thick, dark yellow gel combined with dark yellow plastic-like flakes. MALDI-MS of the crude sample was consistent with two products in the target mass range—one missing the N-terminal K residue, and the other an almost perfect match to the expected isotopic mass calculated above (Figure 4.11 on page 82).

Having confirmed the presence of the desired product in the crude mixture, we decided to lyophilize the gelatinous crude sample in a large volume of $\rm H_2O$ in an attempt to remove TFA and other undesirable impurities which may have persisted from the synthesis, cleavage, and lyophilization in other solvents. After several rounds of lyophilization with excess water, the crude sample was a mixture of white and yellow powder. The new crude sample was successfully dissolved in a solution with final composition as follows: ~ 1.5 mg/mL crude peptide in 1 mL of 50% $\rm H_2O$ / 50% ACN, 200 mM DTT. The purity of the sample was assessed by HPLC as detailed in Figure 4.12 on page 83. Lyophilized fractions from the crude HPLC run were reconstituted in 50% $\rm H_2O/ACN$, either with or without 200 mM DTT (1 hour treatment), and then diluted two-fold with α -CHC matrix. MALDI results were consistent with elution of the product between 18-30 minutes on the HPLC trace (see summary Table 4.1 on page 102 and selected MALDI mass spectra starting with Figure B.1 on page 217).

The apparently broad elution of the desired product, along with low molecular weight impurities, over a 12 minute HPLC window may result from the rather large 6 minute fraction collection times. Therefore, an HPLC run using the same ACN gradient was repeated with a similar preparation of crude, but using narrower 1 minute fraction collection windows. A broad mass of peaks was again apparent between 18-30 minutes (Figure 4.13 on page 84), and MALDI-MS on the 12×1 minute fractions between 18-30 minutes revealed some promising candidates for additional purification (see summary Table 4.2 on page 103 and selected MALDI spectra starting with Figure B.5 on page 221). While the persistence of low molecular weight impurities and the nearly continuous co-elution of the ~2121 g/mol species (missing the N-terminal K) along with the desired product at ~2250 g/mol should be noted, fraction 5 (22-23 minutes) stands out as the most promising with a favorable 2250:2121 species ratio (assuming a similar MALDI ionization potential).

The MALDI-MS of the most promising C3 (HPLC) column-purified TR-09-1 frac-

tion (#5) contains impurities including a major species at ~1096 m/z and the truncated product at ~2121 m/z (Figure B.5 on page 221). In an attempt to improve separation, I repeated the same ACN gradient on a C18 semi-preparative HPLC column with the C3 column-purified fraction #5 as starting material (Figure 4.14 on page 85). A promising set of peaks eluted at ~24-26 minutes from the C18 column, and the run was repeated to collect 15 second fractions in the region of interest (Figure 4.15 on page 86). The best sample purity yet was achieved in fraction #5 from the C18 column, with the target MALDI peak dwarfing the truncated product and most low molecular weight species (see summary Table 4.3 on page 103 and selected MALDI spectra starting with Figure B.11 on page 227).

For the sake of time and yield, it is desirable to avoid a 2-column HPLC purification scheme as described above. Therefore, I loaded a crude TR-09-1 sample directly to the C18 semi-preparative column to assess the HPLC separation that can be achieved (Figure 4.16 on page 87). The run was repeated (Figure 4.17 on page 88) with collection of fractions near the window of optimal purification observed for the C18 column in the two-part purification scheme. It is encouraging that one of the fractions from this single-column purification scheme exhibited the target product as the major peak (see summary Table 4.4 on page 104 and selected MALDI spectra starting with Figure B.15 on page 231). The purest fraction was offset by ~15 seconds relative to the optimal elution in the two-part scheme. The delay may reflect differences in sample viscosity/composition (i.e., C3 column-purified sample would have substantially less impurities than the direct load of crude).

At this stage, it appears that a C18 HPLC column may provide a route to TR-09-1 purification with an optimized ACN gradient. However, I first wanted to verify the purity of fraction # 5 (from the two-column purification scheme) on a C18 analytical column–since this fraction was the purest I had obtained to date and it is desirable to assess the point at which a relatively pure sample elutes from a C18

column. The results were confounded by an impurity present on the C18 analytical column (Figure 4.18 on page 89), and there was no clear candidate peak for elution of the relatively pure sample. Instead of simply repeating the method, I designed a slower ACN gradient for the C18 analytical column in an attempt to achieve better separation of TR-09-1 crude (Figure 4.19 on page 90). However, there were peaks on the tail of the solvent front so I decided to switch to a more conservative starting % ACN and collected fractions for some reasonably well-resolved HPLC peaks near the estimated ~48% ACN elution point for TR-09-1 (Figure 4.20 on page 91). Unfortunately, MALDI-MS results did not show evidence of TR-09-1 in any of the analytical fractions (not shown). I reloaded the fractions to the C18 analytical column and the results did not convincingly demonstrate that I had in fact captured the target peaks with the assumed ~97.5 second delay at 0.48 mL/min (not shown).

Assured delay by HPLC re-calibration allowed me to return to the original challenge—purification of TR-09-1. I decided to aim for bulk purification of TR-09-1 on a C18 semi-preparative column since the C18 results had generally been better than C3, and because another group reported successful purification of similar spitz TMD constructs using a C18 column (153). The entire remaining TR-09-1 stock was dissolved in ~15 mL of 50% ACN/H₂O, 0.1% TFA, 156 mM DTT solution. The mass of crude prior to dissolution was not known, but the latter solution was quite viscous. The first two bulk purification test runs on the C18 semi-preparative column are shown in Figure 4.21 on page 92. The fractions were collected in too narrow a time window in the first run based on the MALDI results (summarized in Table 4.5 on page 104 and see selected MALDI spectra starting with Figure B.19 on page 235). I shifted the fraction collection to a later time window (on the basis of crude viscosity and the latter MALDI results) in the second run, and while the product was more prominent in some of the fractions, some product was present in virtually all of the fractions over the 7 minute collection window (see summary Table 4.6 on page 105 and selected

MALDI spectra starting with Figure B.21 on page 237). The breadth of product elution based on MALDI results is not surprising given the outrageously broad HPLC profiles for crude TR-09-1.

The 2-100% ACN gradient used for the bulk purifications above may ramp too rapidly and result in incomplete separation, especially in the context of a concentrated sample. I tried a set of more conservative gradients in C18 semi-preparative HPLC purifications of the crude—starting at 40% ACN (Figure 4.22 on page 93) or 30% ACN (Figure 4.23 on page 94). Depressingly, TR-09-1 eluted over (practically) the full 26 minute range of collected fractions from the replicate starting at 40% ACN (see summary Table 4.7 on page 105 and selected MALDI spectra starting with Figure B.24 on page 240). Expansion of the collection window in the following run (starting at 30% ACN) revealed a 42 minute elution window (see summary Table 4.8 on page 106 and selected MALDI spectra starting with Figure B.26 on page 242). Some fractions were promising, but this is clearly not a desirable separation.

The ninth fraction (Figure B.26 on page 242) from the HPLC run detailed in Figure 4.23 (page 94) is promising despite the presence of the impurity at ~1096 m/z. I repeated this HPLC gradient for bulk purification of several mL of crude TR-09-1, collecting the equivalent of the ninth fraction and also collecting subsequent eluent up to 52 minutes because of the broad elution profile described above. A subset of these purification HPLC runs are compared with the original run in Figure 4.24 on page 95. All remaining TR-09-1 crude was purified in this fashion. However, it was not possible to achieve purification of sufficient material to perform NMR spectroscopy after several additional rounds of purification (not shown).

4.6 Production And Purification Of A 16-Residue TatA TMD Construct (tr-10-2)

P. stuartii TatA is a substrate of the rhomboid homologue AarA and I targeted a

TM portion of TatA for manual solid-phase peptide synthesis because of the demand on our automated synthesizer and the lack of success producing spitz constructs in an automated context. The Fmoc-based synthesis of the 16-residue TatA construct (ESTIATAAFGSPWQLI), designated TR-10-2, is based on a reported manual synthesis procedure (154) and was produced starting with 100 mg of Fmoc-Ile Wang resin (0.55 mmol/g). Remarkably, all of the colourimetric tests for completion of reactions were successful. The MALDI spectrum of the crude sample reconstituted in ACN produced a promising result that includes prominent peaks for the Na⁺ and K⁺ product adducts (starting with Figure 4.25 on page 96).

To further characterize the purity of the crude TR-10-2 preparation and to assess the prospects for separation, I performed C18 analytical HPLC on the sample (Figure 4.27 on page 98 and Figure 4.28 on page 99). The results were encouraging, with a dominant peak in the traces at 210 nm and 280 nm—the latter consistent with the presence of a W residue in the desired product. Furthermore, the target product was only present in two fractions near the expected elution time of the major peak (see MALDI summary Table 4.9 on page 107 and representative MALDI spectra starting with Figure B.33 on page 249). Impurities persist in the promising fractions but this is not surprising given the width of the HPLC collection windows. Although several attempts were made to collect higher resolution HPLC fractions in the region of interest, a number of problems with HPLC plumbing and possible contamination of HPLC solvents were highly problematic (not shown). Nonetheless, I think this particular TatA construct and manual synthesis are both promising ideas for the production of a spitz homologue.

4.7 Production Of A 37-Residue Gurken TMD Construct (tr-10-1)

Gurken is another *Drosophila* substrate of Rhomboid, and I attempted automated

solid-phase peptide synthesis of a 37 residue construct of gurken (designated TR-10-1) based on the construct outlined in (141). I incorporated a number of partial ¹⁵N-backbone isotope labels (KKRKV[50]RM[50]A[50]HIV[50]F[25]SF[20]PV[50]L[50]-LM[25]LSSL[15]YVL[25]F[10]A[75]A[100]VF[25]ML[50]RKKK, with label % following residues) during the synthesis, which used 0.7 g of Fmoc-Lys(Boc)-Wang resin (loading of 0.19 mmol/g). Colourimetric tests for completed reactions were often not successful, and it is therefore not surprising that crude peptide MALDI-MS characterization did not exhibit any prospective product peaks (not shown). It is also noteworthy that the target product mass ~4405 g/mol is calculated using a procedure similar to that outlined in Figure 4.10 on page 81 for fractional isotope incorporation.

4.8 Production And Purification Of A Spitz Construct By Expression In *E. coli*

Clearly it is difficult to produce spitz TMD constructs by SPPS, and purification by HPLC is even more problematic. Overexpression of a spitz TMD construct in *E. coli* allows for the incorporation of a hexahistidine tag for Ni-affinity purification, a FLAG tag for affinity chromatography, and does not involve the production of truncated products with similar chemical and physical properties (which is observed in SPPS because of failed reactions). Thus, there are options to circumvent HPLC purification or at least simplify the work-up by avoiding truncated species.

A 2.7 mg crude yield was obtained by expression of the construct outlined in Figure 4.29 on page 99 and subsequent Ni-affinity purification. C3 semi-preparative HPLC assessment of the crude produced a promising trace (Figure 4.30 on page 100), and ESI-MS characterization of the major peak ~26 minutes was consistent with the mass of the (N-terminal Met-deficient) unlabelled product (not shown). However, subsequent matching HPLC runs were not consistent, and expression of the ¹³C, ¹⁵N-labelled construct resulted in an extremely poor yield.

A number of expressions were attempted with different strategies for target protein isolation (*i.e.*, use of inclusion bodies), but there were always problems with sample solubility and the apparent presence of oligomers. One MALDI-MS result confirmed that $\sim 50\%$ ¹⁵N incorporation was achieved because of the (accidental) exposure of bacteria to both labelled and unlabelled nutrient sources (Figure 4.31 on page 101). In subsequent spitz expressions there were persistent problems with the presence of dimers, which could not be disrupted by exposure to 8 M urea and/or the reducing agents DTT, TCEP, or β -mercaptoethanol (see representative Figure 4.32 on page 102). Furthermore, MALDI and SDS-PAGE results were both consistent with the formation of higher order oligomers, and this may account for inconsistent elution times in matching HPLC runs. For now, spitz expression constructs are not any more tractable than constructs produced by SPPS and the yields are very low for isotope-labelled expression employing minimal media.

4.9 Conclusions

In this chapter I've described several different strategies and constructs for the production of a spitz or homologous rhomboid substrate with the ultimate objective of determining a high-resolution NMR structure. Although the latter goal was not achieved, it is certainly clear that the production and purification of these TMD constructs is extremely challenging. In light of the difficulties I had assigning resonances in homonuclear spitz TMD NMR spectra (see section 4.4 on page 62), I would strongly suggest that incorporation of isotope labels will be important for future efforts. It is still not clear if cysteine residues are forming disulfide bridges responsible for the extensive oligomerization issues reported in section 4.5 (page 64), but it is not necessarily a bad idea to substitute with Ser as described for the TR-08-2 construct.

It is particularly disturbing that successful HPLC purification of similar spitzbased TMD constructs was reported on a C18 column with no apparent difficulties (153). I have some concern that I often overloaded the column because of the broad traces that were often observed. Conversely, the poor solubility of the samples largely excluded the possibility of loading very high concentrations of crude, but may also account for in-column retention/precipitation. Furthermore, it can hardly be considered desirable to further dilute a sample that required 76× 48-minute HPLC runs for purification (section 4.4 on page 62).

Frustrated by the poor peptide tractability described in this chapter, my remaining spitz-rhomboid studies were performed *in silico* with coarse-grained molecular dynamics simulations (chapter 6 on page 191).

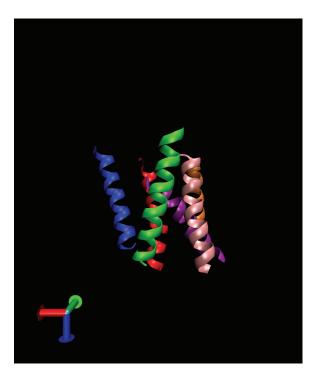


Figure 4.1: A cartoon representation of the TM segments from the ecGlpG structure (PDB: 2IC8; (4)). The top of this representation would represent the extracellular environment, the loops between helices have been excluded, and helix colouring follows the scheme: TM 1 (dark blue), TM 2 (red), TM 3 (green), TM 4 (purple), TM 5 (orange), TM 6 (pink). Note that TM 4, which includes the catalytic serine residue, is protected from the exterior of the protein by the ring of TM helices.

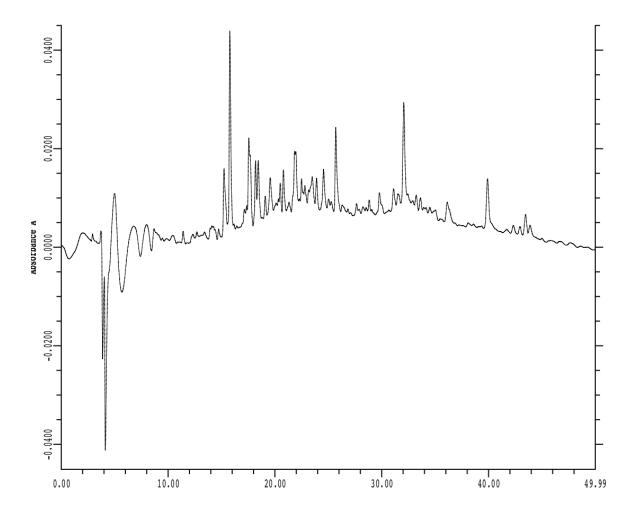


Figure 4.2: 30 μ L of a crude TR-08-2 preparation was loaded to a C18 analytical HPLC column and subject to a 0.8 mL/min gradient: 1) 2% ACN pre-run, 2) +2%/minute ACN up to 100%. Fractions were collected in 5-minute windows between 0 and 50 minutes and this trace is monitored at 210 nm. A preceding blank run with DI-H₂O did not reveal any substantial column-retained impurities (not shown).

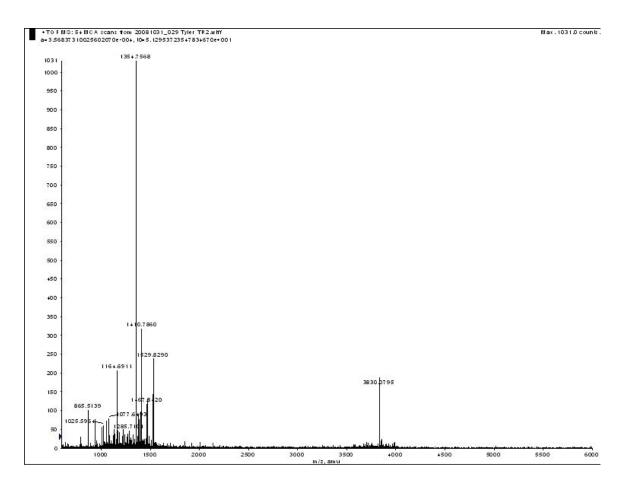


Figure 4.3: The most promising TR-08-2 fraction following several iterative rounds of HPLC optimization produced this MALDI spectrum with a clear candidate peak for the target product ~ 3830 m/z with the monoisotopic theoretical mass ~ 3828 g/mol. There do, however, appear to be some low molecular weight impurities.

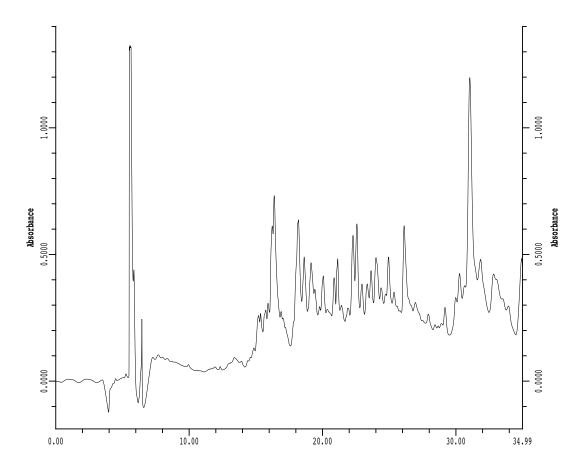


Figure 4.4: This is one of the 76 representative TR-08-2 bulk purification HPLC runs monitored at 210 nm. For each replicate, I collected between 28.5 and 32.3 minutes based on the MALDI results in Figure 4.3 on page 75 for a matching run. The gradient starts at 2% ACN and ramps by 2%/minute.

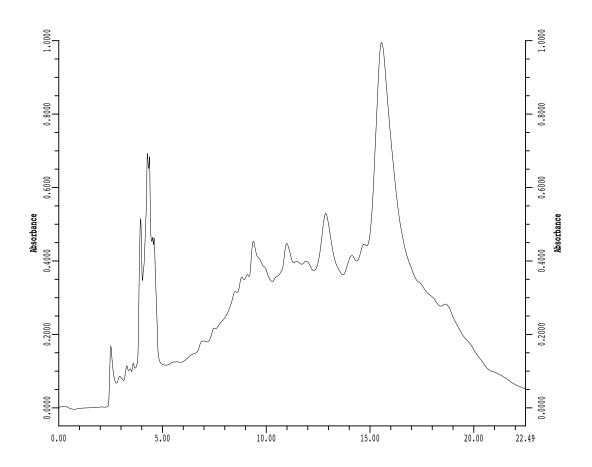


Figure 4.5: Fractions collected as detailed in Figure 4.4 (page 76) were subjected to additional semi-preparative HPLC purification and this is a representative trace monitored at 210 nm. Two fractions were collected between 12.2-14.2 minutes (fraction 1) and 14.8-16.6 minutes (fraction 2).

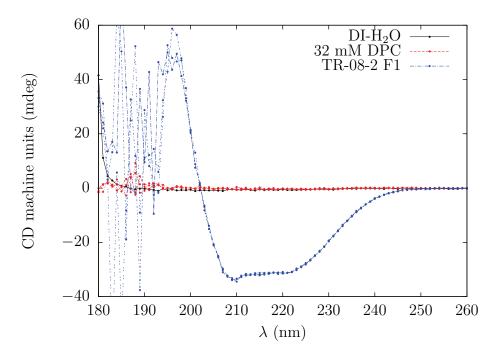


Figure 4.6: Circular dichroism (CD) spectropolarimetry was performed in triplicate for TR-08-2 preparations (100 μ M peptide, 32 mM DPC, pH 5) and a 32 mM DPC control solution at matching pH. A single blank run was also performed with deionized water. Measurements were collected at 37°C with a 20 nm/min scan rate through a 1 mm path length cell. Measured dips near 208 nm and 222 nm are characteristic of α -helical secondary structure.

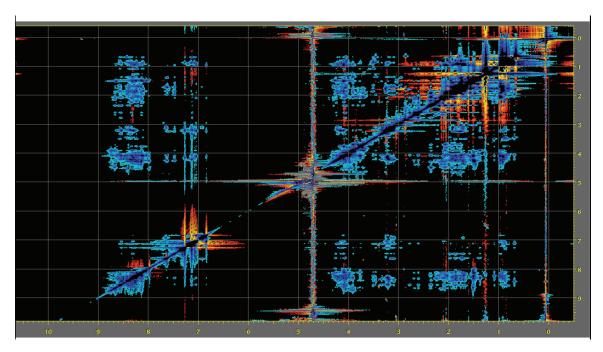


Figure 4.7: Overview of 2D ¹H-¹H NOESY (200 ms mixing time) collected for TR-08-2 on a 700 MHz magnet fitted with a cold probe. Water suppression was performed with excitation sculpting, and the spectrum was collected at 310.15 K.

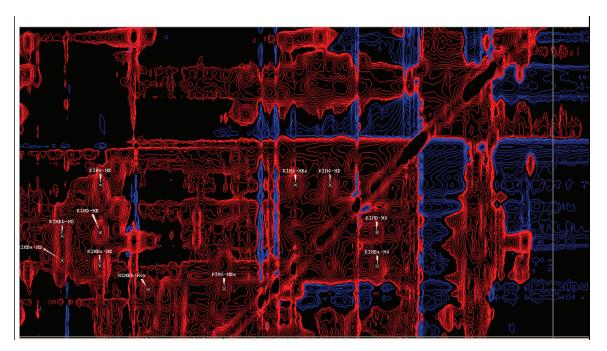


Figure 4.8: A zoom-in view of 2D 1 H- 1 H TOCSY collected for TR-08-2 on a 700 MHz magnet fitted with a cryogenically cooled probe. Water suppression was performed with excitation sculpting, and the spectrum was collected at 310.15 K. Although some assignments are visible in this caption, it is also clear that there is substantial resonance overlap.

KRPRPMLEKASIASGAMSALVFMLFVSLAFYLRK KEKASIASGAMCALVFMLFV KEKASIASGAMCALVFMLFVK

Figure 4.9: Comparing spitz TMD constructs for solid-phase peptide synthesis. The 34-residue construct with $2 \text{ Cys} \rightarrow \text{Ser}$ substitutions (top) was problematic for structure determination by NMR spectroscopy. The middle construct is known to be cleaved by a Rhomboid homologue and was successfully produced by solid-phase peptide synthesis by another group (153). I produced and purified the bottom 21-residue construct because of its similarity to the published construct.

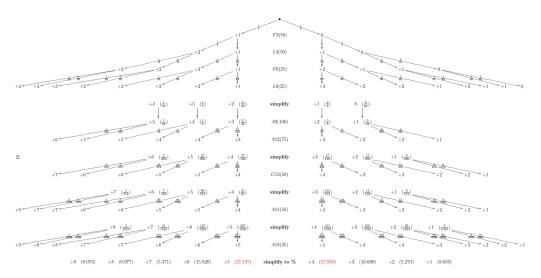


Figure 4.10: There are nine 15 N fractional selective isotope label positions in the tr-09-1 construct, and properly predicting the adjustment to the monoisotopic mass requires a 'tree-branch' probability approach as outlined here. The coupling amino acid (proceeding $C \rightarrow N$ as synthesized) and the isotope % are both indicated near the middle of the row that results from a given coupling. The arrow or parenthetical fractions indicate the proportion of all peptide species that have the resulting atomic mass adjustment (ignoring impurities that form from failed reactions). It is clear that between 4 and 5 atomic mass units should be added to the predicted monoisotopic mass to obtain the most abundant 15 N-enriched species.

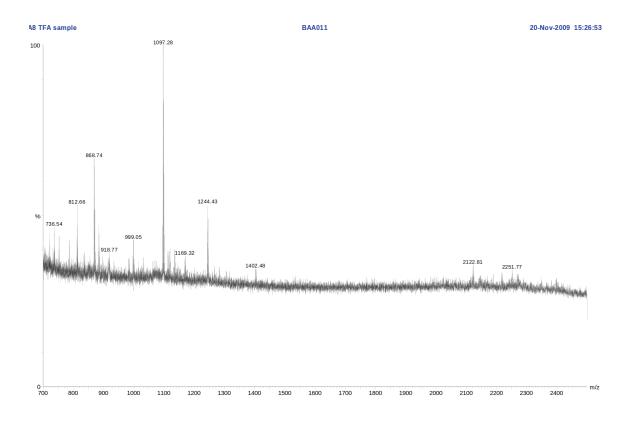


Figure 4.11: A 2.9 mg/mL crude TR-09-1 solution in 100% TFA was diluted with a MALDI matrix solution, and 1 μ L was spotted to a MALDI plate. The mass spectrum was captured in reflectron mode. The peak at ~2252 m/z is consistent with the predicted mass of the desired product while the peak at ~2123 most likely corresponds to the desired product missing the N-terminal K residue.

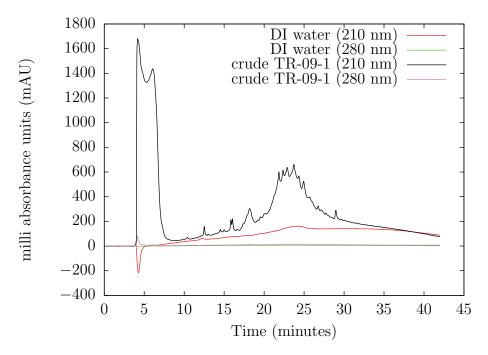


Figure 4.12: 1 mL of 1.5 mg/mL TR-09-1 crude peptide in 50% H₂O / 50% ACN, 200 mM DTT, was loaded to a C3 RP-HPLC semi-preparative column and monitored at 210 nm and 280 nm using a gradient that proceeded from 2-100% ACN in 40 minutes, followed by 100-2% ACN in 2 minutes. The blank was an equivalent volume of deionized water (DI-H₂O). Seven fractions from the crude peptide run were collected in six minute windows—F1 (0-6 minutes), F2 (6-12 minutes), F3 (12-18 minutes), F4 (18-24 minutes), F5 (24-30 minutes), F6 (30-36 minutes), F7 (36-42 minutes). The HPLC dead time at 3 mL/minute is \sim 28 seconds.

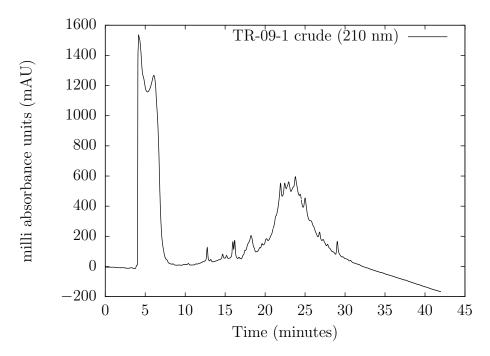


Figure 4.13: 1 mL of a 1.9 mg/mL TR-09-1 crude solution dissolved in 50% H₂O/ACN was treated with 200 mM DTT for 1 hour and loaded to a C3 semi-preparative column. The gradient and flow rate match the conditions detailed in Figure 4.12 on page 83, but in this case narrower 1 minute fractions were collected between 18 and 30 minutes run time. There were no substantial impurities in a preceding blank run with 1 mL of deionized water (not shown).

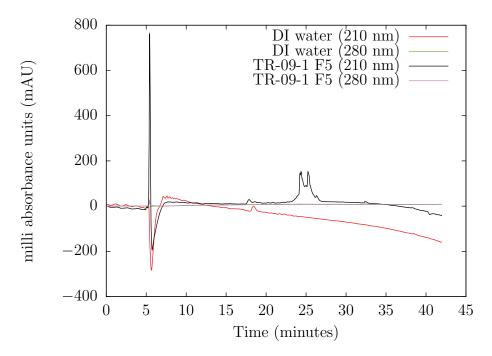


Figure 4.14: 1 mL of a C3 HPLC column-purified fraction (#5) from the run detailed in Figure 4.13 (page 84) was loaded to a C18 semi-preparative HPLC column and elution was monitored at 210 nm and 280 nm during a 3 mL/min gradient involving 2-100% ACN in 40 minutes followed by 100-2% ACN in 2 minutes. A matching volume of deionized water was used as a blank in a preceding HPLC run with the same gradient and flow rate.

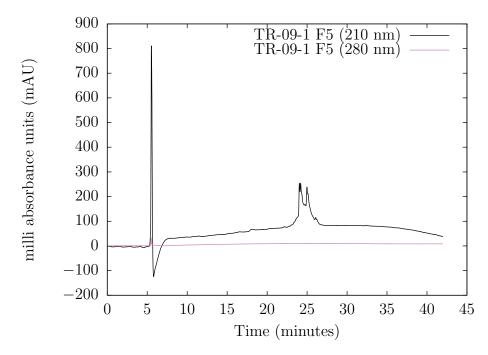


Figure 4.15: A repeat of the C18 semi-preparative HPLC run involving TR-09-1 fraction #5 (from a C3 column) as detailed in Figure 4.14 on page 85. However, in this case 8×15 second fractions were collected starting at 24:16 run time. A preceding blank run with a matching volume of deionized water did not reveal any substantial impurities (not shown).

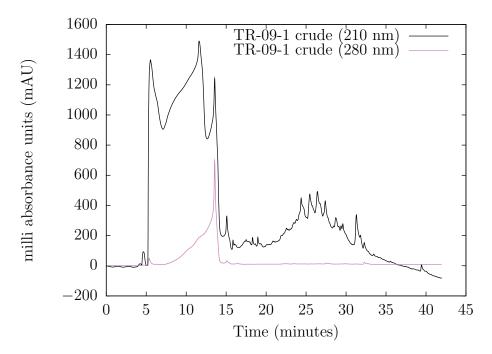


Figure 4.16: 1 mL of 1.3 mg/mL TR-09-1 crude sample in 50% H₂O/ACN and 200 mM DTT was loaded to a C18 semi-preparative column and subjected to the same method described in Figure 4.14 on page 85. A matching volume of deionized water was used in a preceding blank run with no substantial impurities present (not shown).

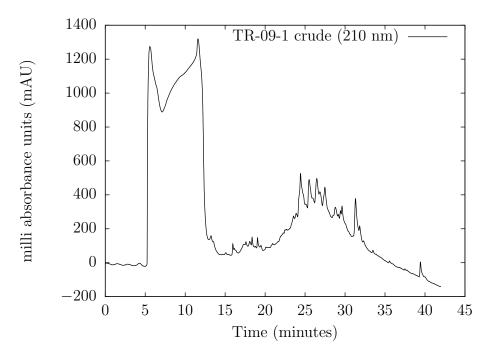


Figure 4.17: 1 mL of 2.1 mg/mL crude TR-09-1 in 50% H₂O/ACN and 200 mM DTT was loaded to a C18 semi-preparative column in a repeat of the run described in Figure 4.16 (page 87), but in this case three fractions were collected to match the promising results from a C18 column summarized in Table 4.3 (page 103): F1 (25:01-25:16), F2 (25:16-25:31), and F3 (25:31-25:46). A matching volume of deionized water was used to confirm the absence of major column impurities in a preceding blank run.

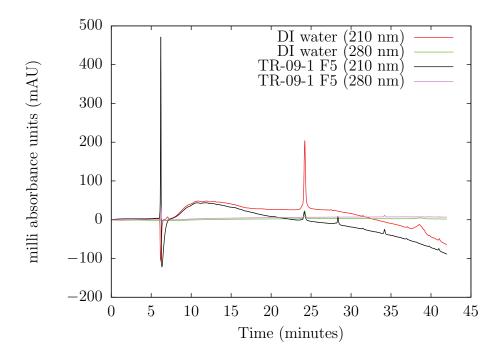


Figure 4.18: 20 μ L of the purest TR-09-1 fraction (# 5 from the two-column HPLC purification scheme) was loaded to a C18 analytical column and subject to an ACN gradient at 0.48 mL/min: 2-100% ACN in 40 minutes, followed by 100-2% ACN in 2 minutes. A matching volume of deionized water was used as a blank in a preceding run, and unfortunately a residual impurity confounds the results of the test trace.

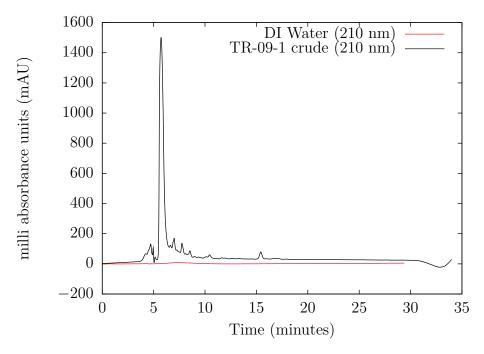


Figure 4.19: A 0.8 mg/mL preparation of TR-09-1 crude in 50% $\rm H_2O/ACN$ and 200 mM DTT was loaded to a C18 analytical column using a 20 $\rm \mu L$ sample loop. The method consisted of a 0.48 mL/min flow rate and a multi-step gradient: 1) Start at 53.9% ACN, 2) 53.9%-66.15% ACN from 0-25 minutes, 3) 66.15%-100% ACN from 25-27 minutes, 4) 100%-53.9% ACN from 27-28 minutes. The peak between 15-16 minutes on the crude trace was collected assuming a ~97.5 second delay based on the flow rate and system plumbing.

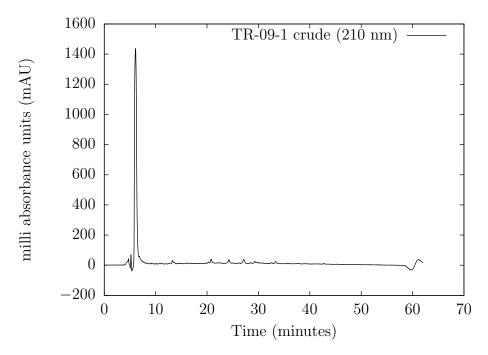


Figure 4.20: The crude TR-09-1 preparation described in Figure 4.19 (page 90) was again loaded to a C18 analytical column using a 20 μ L sample loop, but in this case using a more conservative gradient (still at 0.48 mL/min): 1) 40% ACN pre-run, 2) 40%-66% ACN in 53 minutes, 3) 66%-100% ACN in 1 minute, 4) 100%-40% ACN in 1 minute, 5) Continue monitoring run until ~62 minutes. Three sets of peaks (20-22 minutes, 23-25 minutes, 26-28 minutes) were collected assuming a ~97.5 second delay at this flow rate. A preceding blank run with deionized water demonstrated an absence of impurities on the column (not shown).

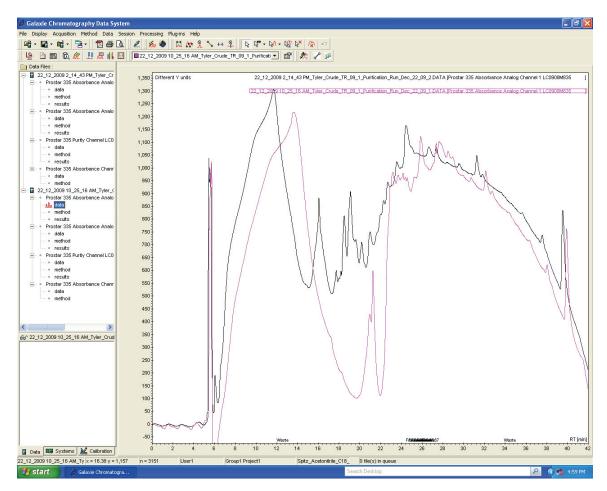


Figure 4.21: The first (pink) and second crude TR-09-1 bulk purification runs monitored at 210 nm. In both cases, 1 mL of crude (dissolved in 50% H₂O/ACN, 0.1% TFA, 156 mM DTT) was loaded to a 2mL sample loop (bottom injector) and subject to a 3 mL/min gradient on a C18 semi-preparative column similar to those described for previous C18 purifications. 7×15 second fractions were collected between 25:10-26:55 from the first replicate, and 14×30 second fractions were collected between 26:55-33:55 from the second replicate.

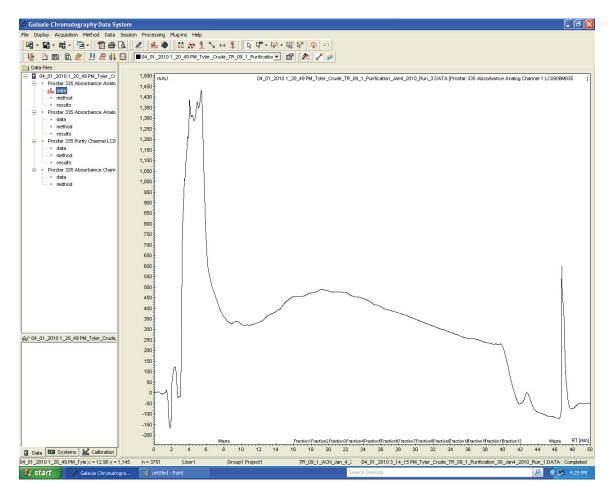


Figure 4.22: $\sim 500~\mu L$ of crude TR-09-1 was loaded to a C18 semi-preparative HPLC column via a 2 mL sample loop (bottom injector). The 3 mL/min gradient involved a few steps: 1) 40% ACN pre-run, 2) 40-75% ACN in 35 minutes, 3) 75-100% ACN in 1 minute, 4) hold 100% ACN for 5 minutes, 5) 100-40% ACN in 1 minute. 2 minute fractions were collected between 16-42 minutes. This trace is monitored at 210 nm, and a matching run with deionized water demonstrated an absence of column-retained impurities.

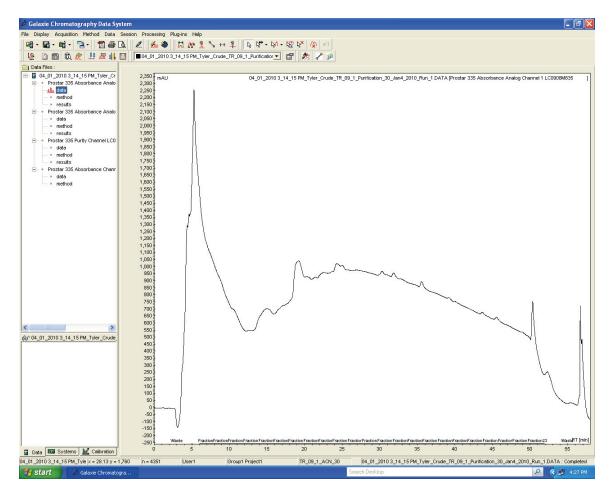


Figure 4.23: 1 mL of crude TR-09-1 was loaded to a C18 semi-preparative HPLC column via a 2 mL sample loop (bottom injector). The 3 mL/min gradient was modified from the previous run (Figure 4.22 on page 93) to start at a lower % ACN: 1) 30% ACN pre-run, 2) 30-75% ACN from 0-45 minutes, 3) 75-100% ACN from 45-46 minutes, 4) hold 100% ACN from 46-51 minutes, 5) 100-30% ACN from 51-52 minutes. This trace is monitored at 210nm.

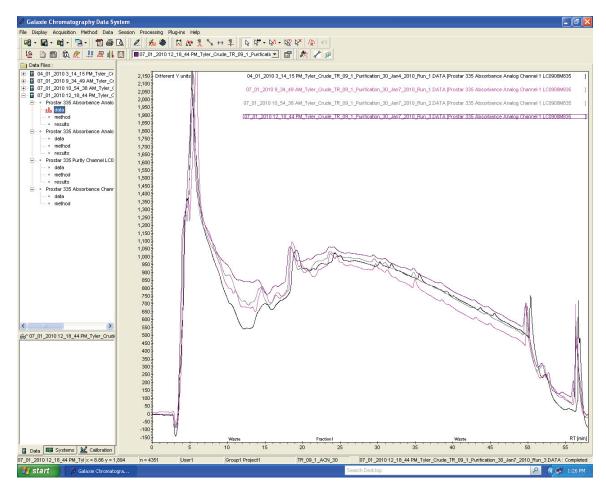


Figure 4.24: 1 mL of crude TR-09-1 was loaded to a C18 semi-preparative HPLC column and subjected to the same gradient described in Figure 4.23 on page 94. Based on the latter (original) HPLC run, the new purification runs targeted collection of the promising fraction that elutes between 22-24 minutes, but the remaining eluent up to 52 minutes was also collected because the product exhibits a very broad elution profile (see Table 4.8 on page 106). The 210 nm traces shown here compare the original HPLC run (in black) with the more recent bulk purifications. Unfortunately, the more recent runs accidentally employed a 1 mL sample loop instead of the 2 mL loop used in the original run, causing an earlier elution profile.

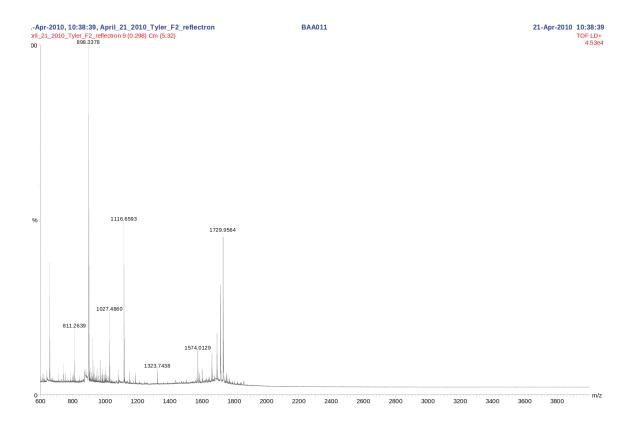


Figure 4.25: The TR-10-2 peptide construct was cleaved from the synthesis resin, lyophilized with excess water, and the crude was reconstituted with 50% ACN/ α -CHC matrix and the displayed reflectron-mode MALDI spectrum was collected. The target product should be ~1691 g/mol, and the large peak at ~1729 g/mol is almost certainly the K⁺ adduct. While this is a promising result for the first attempt at manual solid-phase peptide synthesis in our lab (and for a zoom-in version see Figure 4.26 on page 97), there are certainly some low molecular weight impurities.

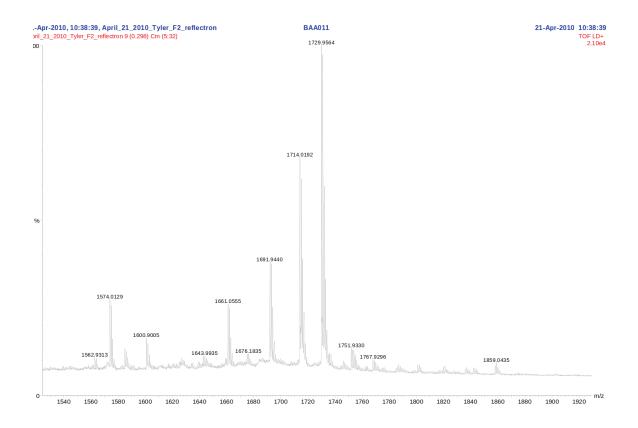


Figure 4.26: A zoom-in view of the reflectron-mode MALDI spectrum detailed in Figure 4.25 on page 96. The TR-10-2 target product is ~ 1691 m/z, while the Na⁺ and K⁺ adducts are also clearly visible ~ 1714 m/z and ~ 1729 m/z respectively.

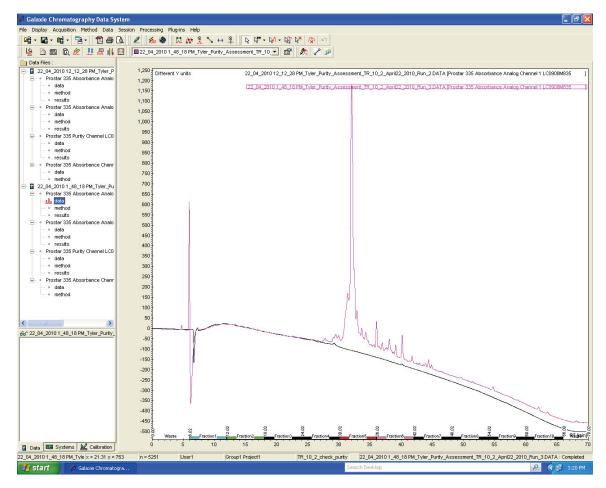


Figure 4.27: A crude preparation of TR-10-2 dissolved in ACN was loaded to a C18 analytical column via a 20 μ L sample loop (top injector) and subject to a 0.48 mL/min gradient: 1) 2-100% ACN in 60 minutes, 2) hold 100% ACN for 5 minutes, 3) ramp back 100-2% ACN in 1 minute. The trace shown here was monitored at 210 nm for the test run (pink) and a matching blank run with DI-H₂O (black) and fractions were collected in 6 minute windows between 6 and 66 minutes. The trace monitored at 280 nm is shown in Figure 4.28 on page 99.

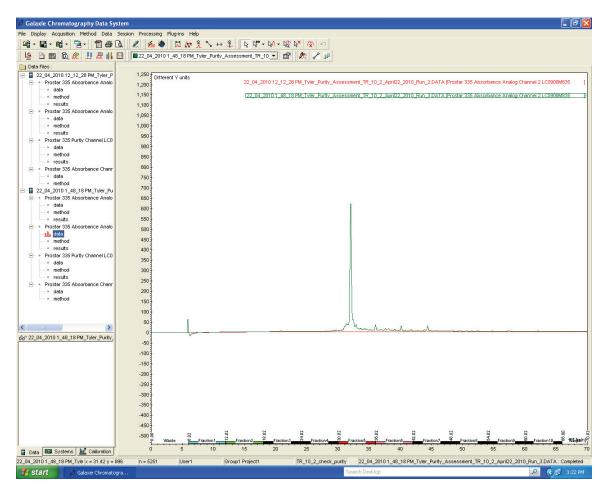


Figure 4.28: This is the TR-10-2 C18 analytical HPLC purity assessment run detailed in Figure 4.27 (page 98) monitored at 280 nm for the crude peptide (green) and the DI- H_2O blank (red).

$\mathrm{NH_{2}\text{-}MSGSHHHHHHGSSGENLYFQSLEYKEIDNTYLPKRPRPMLEKASIASGA}$ $\mathrm{MCALVFMLFVCLAFYLRFEQRAAKKDYKDDDDK\text{-}COO^{-}}$

Figure 4.29: Expected (82-residue) spitz fusion peptide produced by expression in pEXP5-NT/TOPO vector with TEV protease cleavage site highlighted in red. There is an N-terminal His tag and a C-terminal FLAG tag. The N-terminal Met is normally removed during production, shifting the expected average isotopic mass from \sim 9524 g/mol to \sim 9393 g/mol.

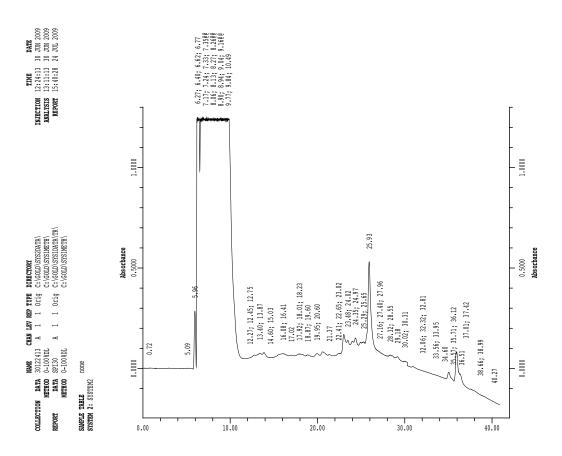


Figure 4.30: 250 μ L of ~1 mg/mL semi-crude (Ni-purified) expressed spitz construct in 50% H₂O/ACN was loaded to a C3 semi-preparative HPLC column and subject to a 2 mL/min gradient that ramps from 0-100% ACN at 2.5%/min. The trace shown here is monitored at 210 nm, and encouragingly the major peak ~26 minutes was confirmed by ESI-MS to correspond to a species with average molecular weight consistent with the target for the construct (not shown).

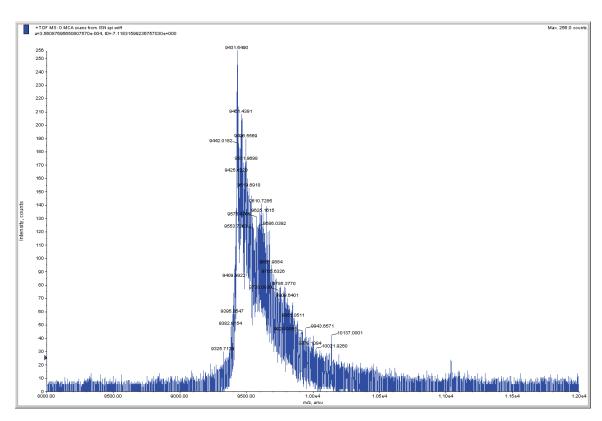


Figure 4.31: MALDI spectrum for the expressed spitz construct detailed in Figure 4.29 on page 99. The mass of the major peak is roughly consistent with a $\sim 50\%$ $^{15}{\rm N}$ incorporation. This is sensible as the bacteria were (accidentally) exposed to both isotopically enriched and unenriched nutrient sources.

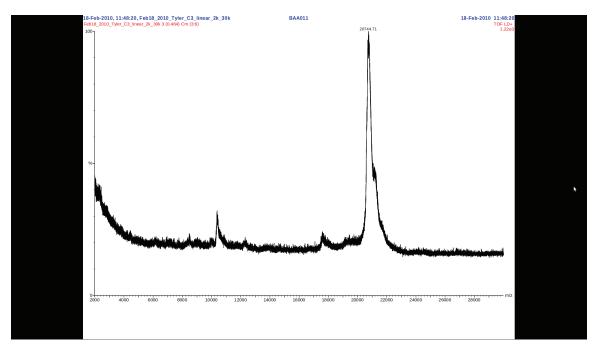


Figure 4.32: A crude expressed spitz construct dissolved in 50% $\rm H_2O/ACN$ (0.1% TFA) was treated with immobilized TCEP, separated from the reducing agent by centrifugation, diluted two-fold with sinapic acid (matrix solution), and the resulting linear-mode MALDI spectrum is shown here. After accounting for the linear-mode calibration, a potential dimer peak ~20.6 kDa dwarfs a peak consistent with the monomer ~10.3 kDa.

Table 4.1: Summary of MALDI-MS results for the HPLC fractions collected during the TR-09-1 crude peptide run described in Figure 4.12 on page 83. Samples were diluted two-fold in α -CHC matrix and the spectra were collected in reflectron mode.

Fraction#	HPLC window (minutes)	200 mM DTT	Peak ~2250 (m/z)
F2	6-12	yes	_
F3	12-18	yes	_
F4	18-24	yes	+
F5	24-30	yes	+
F6	30-36	yes	_
F2	6-12	no	_
F3	12-18	no	_
F4	18-24	no	+
F5	24-30	no	+
F6	30-36	no	_

Table 4.2: 1 minute fractions collected during the TR-09-1 crude HPLC run detailed in Figure 4.13 (page 84) were combined with α -CHC matrix and MALDI spectra were collected without DTT treatment in reflectron mode. A '+' in the table indicates the presence of the desired product (~2250 m/z), which clearly exhibits a broad elution profile.

Fraction#	HPLC window (minutes)	Peak $\sim 2250 \text{ (m/z)}^a$
F1	18-19	_
F2	19-20	_
F3	20-21	- (2218 present)
F4	21-22	- (2217 present)
F5	22-23	++ (2250 major/2121 minor)
F6	23-24	+ (2122 major)
F7	24-25	+ (2122/2251 minor)
F8	25-26	+ (2121/2250 minor)
F9	26-27	+ (2122 present)
F10	27-28	+ (2121 present)
F11	28-29	+ (2122 present)
F12	29-30	+ (2122 present)

 $^{^{}a}$ while the focus is on peaks near the target mass, low molecular weight impurities do persist in many of these fractions

Table 4.3: A C3 HPLC column-purified fraction was further purified on a C18 column and 15 second fractions were collected in a region of interest on the trace detailed in Figure 4.15 on page 86. The latter fractions were diluted two-fold with α -CHC matrix and MALDI spectra were collected in reflectron mode. This table summarizes the results using a '+' to indicate the presence of the desired spitz construct ~2250 m/z. Fraction 5 represents the highest purity TR-09-1 sample obtained to date.

Fraction#	HPLC window (minutes)	Peak $\sim 2250 \text{ (m/z)}^a$
F1	24:16-24:31	_
F2	24:31-24:46	-(2089 present)
F3	24:46-25:01	-(2090 present)
F4	25:01-25:16	+ (1097>>2249>2121)
F5	25:16-25:31	$++ (2249 \text{ major}^b >> 1097 > 2121)$
F6	25:31-25:46	+
F7	25:46-26:01	+ (2250 minor)
F8	26:01-26:16	+ (2122 / 2250 minor)

 $^{^{}a}$ while the focus is on peaks near the target mass, low molecular weight impurities do persist in many of these fractions

^bNa⁺ and K⁺ adducts also present

Table 4.4: Crude TR-09-1 was directly loaded to a C18 semi-preparative HPLC column and fractions were collected as described in Figure 4.17 (page 88). The fractions were diluted two-fold with α -CHC matrix and MALDI spectra were collected in reflectron mode. A '+' is used to indicate the presence of the desired TR-09-1 peptide \sim 2250 m/z in this summary table.

Fraction#	HPLC window (minutes)	Peak ~2250 (m/z)
F1	25:01-25:16	+(1096>>2250)
F2	25:16-25:31	+(1096>>2250)
F3	25:31-25:46	++ (2250>1096)
Control^a		no major peaks

 $[^]a50\%$ H₂O/ACN, 0.1% TFA combined with α -CHC matrix

Table 4.5: TR-09-1 fractions were collected from the first replicate HPLC run detailed in Figure 4.21 on page 92, and diluted two-fold with α -CHC matrix prior to collection of MALDI spectra in reflectron mode. A '+' in the table reflects the presense of the desired product ~2250 m/z.

Fraction#	HPLC window (minutes)	Peak ~2250 (m/z)
F1	25:10-25:25	_
F2	25:25-25:40	+ (minor)
F3	25:40-25:55	+ (minor)
F4	25:55-26:10	- (2218 present)
F5	26:10-26:25	_
F6	26:25-26:40	+ (minor)
F7	26:40-26:55	+ (minor)
R	recovery ^a	+

^asolution contains remaining eluent in the range of 22-30 minutes

Table 4.6: TR-09-1 fractions were collected from the *second* replicate HPLC run detailed in Figure 4.21 on page 92, and diluted two-fold with α -CHC matrix prior to collection of MALDI spectra in reflectron mode. A '+' in the table reflects the presence of the desired product ~2250 m/z.

Fraction#	HPLC window (minutes)	Peak $\sim 2250 \text{ (m/z)}$
F1	26:55-27:25	+
F2	27:25-27:55	+
F3	27:55-28:25	+
F4	28:25-28:55	++ (2121 present)
F5	28:55-29:25	++ (2121 present)
F6	29:25-29:55	+ (2121 present)
F7	29:55-30:25	+ (2121 present)
F8	30:25-30:55	++ excellent (2121 present)
F9	30:55-31:25	++ excellent (2121 present)
F10	31:25-31:55	++ excellent (2121 present)
F11	31:55-32:25	- (low ion count)
F12	32:25-32:55	+ (low ion count) (2122 present)
F13	32:55-33:25	+ (low ion count)
F14	33:25-33:55	+ (low ion count)

Table 4.7: Fractions collected from a bulk purification of TR-09-1 (detailed in Figure 4.22 on page 93) were diluted two-fold with α -CHC matrix and this table summarizes the reflectron-mode MALDI spectra. A '+' in the table reflects the presence of the desired product ~2250 m/z.

Fraction#	HPLC window (minutes)	Peak $\sim 2250 \text{ (m/z)}^a$
F1	16-18	+ (2250 major)
F2	18-20	+
F3	20-22	+
F4	22-24	+ (2249 minor)
F5	24-26	+
F6	26-28	+
F7	28-30	+
F8	30-32	+
F9	32-34	+
F10	34-36	_
F11	36-38	+ (minor)
F12	38-40	+ (minor)
F13	40-42	+

^amost of the fractions had rather low ion counts

Table 4.8: Fractions collected from a bulk purification of TR-09-1 (detailed in Figure 4.23 on page 94) were diluted two-fold with α -CHC matrix and this table summarizes the reflectron-mode MALDI spectra. A '+' in the table reflects the presence of the desired product ~2250 m/z.

Fraction#	HPLC window (minutes)	Peak ~2250 (m/z)
F1	6-8	_
F2	8-10	_
F3	10-12	+ (2249 minor)
F4	12-14	_
F5	14-16	_
F6	16-18	_
F7	18-20	+ (2249 minor)
F8	20-22	+ (2250 minor)
F9	22-24	+ excellent
F10	24-26	+
F11	26-28	+
F12	28-30	$+^a$
F13	30-32	+
F14	32-34	$+^{b}$
F15	34-36	+
F16	36-38	+
F17	38-40	+
F18	40-42	+ (2250 major)
F19	42-44	+
F20	44-46	+ excellent
F21	46-48	+ excellent
F22	48-50	+
F23	50-52	+

 $[^]a$ substantial variety of peaks in ~ 2000 m/z range

^bsubstantial variety of peaks in ~2000 m/z range

Table 4.9: TR-10-2 fractions collected from the HPLC run detailed in Figure 4.27 (page 98) were diluted two-fold with α -CHC matrix and this table summarizes the collected reflectron-mode MALDI results. A '+' symbol is used to indicate the presence of the target product ~1691 m/z (or one of its salt adducts).

Fraction#	HPLC window (minutes)	Peak ~1691 (m/z)
1	6-12	_
2	12-18	_
3	18-24	_
4	24-30	_
5	30-36	+
6	36-42	+
7	42-48	_
8	48-54	_
9	54-60	_
10	60-66	_

Chapter 5

FGFR3 Simulation

5.1 Fibroblast Growth Factor Receptors

Fibroblast growth factor receptors (FGFRs) are a family of four FGF-activated receptor tyrosine kinase (RTK) transmembrane (TM) glycoproteins (155, 156, 157). All FGFRs have three extracellular immunoglobulin (Ig)-like domains, and alternative splicing of the proximal Ig-like domain in FGFRs 1-3 produces receptor isoforms with different ligand-binding specifities (158, 159, 160). The signaling complexity of the FGFR family is further compounded by the existence of at least 19 different FGFs (158, 161). Ligand-binding at the proximal Ig-like domain likely stabilizes the active dimer, with rearrangements in the TM and cytoplasmic tyrosine kinase domains driving autophorphorylation and signal transduction. FGFRs are of medical interest because many skeletal dysplasias and cancers are associated with mutations in FGFRs 1-3.

5.2 Achondroplasia: Specific Relevance Of FGFR3

Achondroplasia, the most common form of human dwarfism, was mapped to the short arm of chromosome 4 (162), and shortly thereafter a G380R mutation resulting from a transition or transversion in the FGFR3 gene was specifically identified as the major cause of the phenotype (163, 164). Indeed, G380R (in the FGFR3 TM

domain) is the underlying cause of achondroplasia in ~99% of cases (163, 164, 165). Achondroplasia is autosomal dominant with complete penetrance (166, 167), and in 80-90% of cases results from a spontaneous paternal germ line mutation (there is a correlation with increased paternal age) (168, 169, 170). While intelligence is normal in individuals with achondroplasia, there is increased mortality in the first four years of life and in the late fourth to fifth decades of life (171).

There is experimental evidence for increased signaling by the mutant FGFR3 with resultant negative regulation of bone growth (172). However, free energies of dimerization measured by fluorescence resonance energy transfer (FRET) in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) for wild-type and G380R TMD peptides match within the bounds of experimental uncertainty (173). Neutron diffraction and oriented circular dichroism spectroscopy indicate a 5 Å shift of the mutant peptide away from a POPC bilayer center (11). There appears to be a conceptual disconnect between the latter perturbation in FGFR3 mutant membrane topology relative to WT and the similar dimerization propensity to dimerize. Because of the medical relevance, there is a substantial motivation to gain insight into the influence of the G380R mutation on the dimerization properties of FGFR3.

In this chapter I describe and analyze coarse-grained molecular dynamics (CG-MD) simulations of the FGFR3 WT and G380R mutant TMDs in lipid bilayers. The analysis involves simulations for the WT homodimer, the heterodimer (because achondroplasia is autosomal dominant), and the mutant homodimer (section 5.4 on page 111). In section 5.5 (page 126) I focus specifically on the properties of the lipid bilayer proximal and distal to the peptide TMDs. Monomer simulations were also performed to control for the behaviour of the individual peptide constructs, and a preliminary analysis of these results is presented in section 5.6 (page 134).

5.3 Coarse-Grained Simulations And Glycophorin A Control

Ideal α -helical atomistic starting structures for FGFR3 constructs were built in PyMOL (174) based on the primary sequences of published experimental constructs (11) and the amino acids were coarse-grained as described previously for lipids (175). An approximate 4:1 mapping of heavy atoms (i.e., non-hydrogen atoms) to CG particles is performed to produce several specific particle types: polar (P), mixed polar/apolar (N), hydrophobic apolar (C), and charged (Q). A few additional particle subtypes allow fine tuning of Lennard-Jones potentials to reflect hydrogen-bonding propensities. Details of the amino acid to CG particle mapping process have been described elsewhere (176, 177). Lipid and water molecules were parametrized as described previously (175), and all simulations were performed using GROMACS (178). The MARTINI force field (179, 180) was employed for all simulations except for the FGFR3 monomer simulations (section 5.6.2 on page 135) which employ the Bond force field (176). Lennard-Jones interactions were shifted to zero between 9 and 12 Å, and electrostatics were shifted to zero between 0 and 12 Å. All simulations were performed at constant temperature, pressure, and number of particles. The temperature coupling of system components was performed independently for each component using the Berendsen algorithm at 323 K (181). The system pressure was semiisotropically coupled in the x, y, and z directions using the Berendsen algorithm. The time step for integration was 40 fs. VMD (182) was used for visualization and MDAnalysis (183) for parsing of trajectories. Parallel simulations and analyses were performed for glycophorin A (GpA) as described for previous CG-MD simulations (184). GpA is a well-characterized single-pass TMD protein which serves as a control for FGFR3—a protein for which there is no high-resolution structure available.

It should be noted that there are, of course, limitations to CG-MD simulations. The coarse-grained nature of the system provides improved performance at the cost of reduced accuracy and reduced similarity to the true biological system. Molecules diffuse at least four times faster than they would in a more realistic atomistic context, the involvement of water in many processes is often not well characterized because of the much larger size of CG-MD water particles, there are no hydrogen atoms or hydrogen bonds (only crude approximations and restraints), and the screening of electrostatic interactions is only approximated and frequently cut-off in a crude fashion.

5.4 Analysis Of FGFR3 And GpA *Dimer* Trajectories

5.4.1 Tracking The Distance Between Helices In A Trajectory

The initial separation between glycophorin A (GpA) and FGFR3 helices in the dimer simulations was ~55 Å, the same value used in previous GpA coarse-grained (CG) molecular dynamics (MD) simulations (184). Tracking the separation between helices in the POPC bilayer is useful for testing a number of properties. Perhaps most obvious is the assessment of the rate of dimer formation—if the separation between helices decreases and reaches the final dimer interhelix separation rapidly then dimer formation occurs quickly for a given construct. The stability of the dimer can also be assessed because increased interhelix separation after initial dimer formation may indicate that the dimer has dissociated, and frequent association and dissociation events may indicate a weak dimerization affinity. Performing many replicate simulations (see Table 5.1 on page 189) may also allow for the determination of whether the rates of dimerization and the dimer stability are consistent for a given dimer construct or vary stochastically between simulations.

My specific strategy was to track the closest interhelix approach using only C_{α} particles from each of the peptides. In each frame of a given simulation, a dis-

tance matrix for all possible interhelical C_{α} combinations was calculated with the minimum value defined as the closest approach distance. Although I coded a few MDAnalysis-dependent (183) python functions for these calculations, the most efficient function is closest_contacts_efficient() from the python module/library dimer_geometric_tools.py D.17 on page 356.

The closest interhelix approach is monitored during the first replicate trajectory of the GpA wild-type (WT) homodimer control condition in Figure 5.1 on page 138. Dimerization occurs very rapidly and the dimer is stable once formed. In contrast, dimerization takes $> 1\,\mu s$ in the FGFR3 WT replicate 3 simulation (Figure 5.2 on page 139), although the dimer is still stable once formed. The ninth FGFR3 heterodimer replicate simulation requires almost $4\,\mu s$ for dimerization (Figure 5.3 on page 139), but the dimer is again stable once formed. The second FGFR3 mutant homodimer replicate exhibits extremely fast dimerization and the dimer remains stable for the duration of the simulation (Figure 5.4 on page 140).

The outlined results may lead the reader to believe that the FGFR3 heterodimer forms more slowly than the WT or the mutant homodimer. However, it is important to consider the closest approach distance results across all of the replicate simulations (Figure 5.5 on page 141). It is clear from the latter plot that the variation in dimerization rates within any of the FGFR3 constructs is considerable. Thus, there appears to be a stochastic component to the rate of dimerization with no particular trend for FGFR3 WT, heterodimer or mutant homodimer conditions. However, in all cases, GpA and FGFR3 dimers are stable once formed in the self-assembled CG POPC bilayers used in these simulations since no dissociations were observed in 34 replicate simulations. The varied dimerization rates are consistent with the previously reported time required for WT GpA helix association in DPPC—varying from 0.5 to 3 μ s (with a 60 Å initial separation) (185). Note that the fifth GpA replicate simulation does not exhibit dimer dissocation—one of the helices adopted an orienta-

tion perpendicular to the membrane normal (and interacted with the other TM helix) before finally returning to a transmembrane orientation and forming a stable dimer.

5.4.2 Relative Helical Motion

All GpA and FGFR3 dimer constructs are stable once formed, but there is a stochastic component to the rate of dimerization. What are the helices doing during the variable simulation time prior to dimerization? One way to simplify the problem is to look at the motion of one helix in the fixed reference frame of the other. Specifically, in each frame of a (GpA or FGFR3) dimer simulation the coordinates of the geometric center of the first helix were subtracted so that its position was always at the origin. For consistency, the same subtraction per frame was applied to the coordinates of the geometric center of the second helix. Tracking the motion of the second helix in this fashion has been done previously for GpA dimer simulations in DPPC (184). The authors highlight one caveat—the dimerization interface will not be specific because the reference helix is free to rotate about its axis at the origin (as long as the geometric center is fixed).

The MDAnalysis-based relative_helical_motion() python function I wrote in the dimer_geometric_tools module D.17 (on page 356) performs the described adjustment of the trajectory reference frame and printing of new helix 2 coordinates. In the case of the first GpA control replicate, helix 2 follows a relatively straightforward path from its starting position to dimerization with helix 1 (Figure 5.6 on page 142). The case for FGFR3 wild-type replicate 7 is more convoluted, with helix 2 moving through several periodic boundaries before dimerizing with a mirror image of the reference helix (Figure 5.7 on page 142). The ninth heterodimer FGFR3 replicate requires nearly $4 \mu s$ for dimerization (Figure 5.3 on page 139) and accordingly helix 2 moves through a large distance, including many periodic boundaries, before dimerization with a mirror image of helix 1 (Figure 5.8 on page 143). The rapid dimerization of the second FGFR3 mutant homodimer replicate (Figure 5.4 on page 140) is con-

sistent with the straightforward path followed by helix 2 for dimerization with helix 1 in this simulation (Figure 5.9 on page 143). Thus, the variety of dimerization rates is reflected in the relative 'wandering' motions of the helices.

5.4.3 Helix Crossing Angle

Sections 5.4.1 (page 111) and 5.4.2 (page 113) demonstrate a wide variety of helix dimerization rates and wandering distances before dimerization, but a stable dimer once it has formed. The observations are consistent with an unbiased simulation setup. Had the helices been placed too close together at the start of the simulation, the dimerization rates would likely have been more consistent with no opportunity for relative wandering motion. Given the formation of stable and unbiased dimers we now focus on the details of the dimer proper.

It has been well established that GpA dimers exhibit a strong preference for righthanded helix crossing angles from experimental high-resolution structures (186, 187) and computational studies in DPPC (184). The first GpA replicate simulation in POPC is consistent with this behaviour (Figure 5.10 on page 144). There is currently no high-resolution experimental structure of the FGFR3 TM domain (we employ an ideal α -helix), so it is important to establish that the dimer configuration for the GpA control is consistent with previous experimental and computational findings. Unlike GpA, the first FGFR3 WT homodimer replicate exhibits dimerization with a bimodal helix crossing angle distribution, and perhaps a slight preference for a left-handed crossing angle (Figure 5.11 on page 145). The ninth replicate FGFR3 heterodimer simulation appears to exhibit a preference for a right-handed helix crossing angle (Figure 5.12 on page 146), but there is a small number of sampled dimer frames because this trajectory required so long for dimerization (Figure 5.3 on page 139). The first replicate of the FGFR3 mutant homodimer construct exhibits a bimodal helix crossing angle distribution (Figure 5.13 on page 147) similar to that observed for the wild-type replicate above.

While there is value in analyzing replicate simulations on an individual basis, it should also be clear that it is important to perform many replicates and study the merged results to establish overall trends. In particular, the helix crossing angle results merged over all the replicate trajectories indicate that all FGFR3 constructs (including heterodimer) exhibit bimodal helix crossing angle distributions, in contrast to GpA which exhibits an overall preference for a right-handed helix crossing angle (Figure 5.14 on page 148). The bimodal helix crossing angle distribution for FGFR3 constructs might be explained by alternation between configurations as the helices oscillate closer and farther apart in the dimer. However, helix crossing angles were independent of the closest interhelical approach between C_{α} particles for all FGFR3 constructs (Figure 5.15 on page 149).

5.4.4 Correlated Helical Motion

There is stochastic wandering prior to dimerization in the GpA and FGFR3 replicate trajectories, and the dimers are stable once formed, but the helix crossing angle distribution for all FGFR3 constructs is curiously bimodal. It is not clear how the FGFR3 helices move relative to one another in the context of the dimer since both left- and right-handed crossing angles are sampled in a given trajectory. However, since the dimers are stable we would still expect them to move together in the POPC bilayer. To test for coordinated helical motion, I first tracked the Z coordinate (along the bilayer normal) of the geometric center of the C_{α} particles of each helix relative to the center of the bilayer. The center of the bilayer was defined as the average of the center of mass coordinates of the two leaflet phosphate CG particle populations, and the MDAnalysis-dependent python function I wrote for this calculation (geo_Z_tracking_relative_to_bilayer()) is stored in the python library dimer_geometric_tools.py D.17 on page 356. An example of the results of this analysis for the first GpA replicate simulation are shown in Figure 5.16 on page 150. While there is certainly evidence for coordinated excursions of the helical geometric

centers away from the center of the bilayer, it is rather cumbersome to quantify the degree of coordinated motion by inspection.

To quantify the degree of coordinated helical motion in GpA and FGFR3 dimer simulations, I calculated the absolute correlation coefficient (|R|) between the Z coordinates of the geometric centers of the helices before and after dimerization. The python split_Z_file() function in the dimer_geometric_tools.py library (D.17 on page 356) splits the helix geometric center Z coordinates into pre- and post-dimerization files and produces the corresponding linear correlation coefficients. The results for individual replicates are summarized in Figure 5.17 on page 151, and for merged data that includes all replicates per condition in Figure 5.18 on page 152. For all constructs, as expected, helical motion is more strongly correlated following dimerization. The larger correlations observed for GpA dimers relative to FGFR3 constructs is consistent with previous results indicating that GpA forms much stronger dimers than FGFR3 (188).

5.4.5 Identification Of Predominant Interhelical Contacts

The unbiased GpA and FGFR3 simulations exhibit formation of stable dimers which move together in the membrane. In addition, FGFR3 has no apparent preference for left- or right-handed helix crossing angles. However, these are fairly broad properties, and I would now like to focus on more specific aspects of the dimer configuration. Which residues feature most prominently at the dimer interface for FGFR3? Without a high-resolution structure of the FGFR3 TM domain available, the identification of important dimer interface residues via simulation may provide insight into the molecular mechanism of pathology in achondroplasia and other skeletal dysplasias.

I devised a simple method to parse a CG-MD trajectory for the predominant interhelical dimer contacts. For each frame of a simulation (if the helices are within 7 Å), a distance matrix of all possible interhelical C_{α} - C_{α} combinations is calculated. The five smallest distances for each frame are recorded along with the corresponding residue identifiers. The probability for each residue to appear in the five closest dimer contacts is then calculated based on the total number of these contacts in which the residue is found divided by the total number of contacts (which is 5× the number of dimer frames in the simulation). Separate close contact probabilities are calculated for matching residues in each helix in order to assess symmetry. I have written MDAnalysis-dependent python functions to calculate the residue close contact probabilities for each helix in: a single GpA trajectory (top_five_closest_residues_GpA()), a single FGFR3 trajectory (top_five_closest_residues_FGFR3), and aggregate results for each of the FGFR3 constructs across all replicate trajectories (merged_top_five_FGFR3()). The functions are all stored in the library module D.17 (dimer_geometric_tools.py) on page 356. In the case of aggregate probabilities, the head script (analyze_FGFR3_dimer_simulations.py) D.15 on page 338 must also call a second library function (parse_overall_FGFR3_top_five_data()) to parse the larger set of data.

The results of the residue close contact probability analysis for the first replicate GpA simulation are plotted in Figure 5.19 on page 152. The predominant residues are representative of the other GpA replicates with G79, G83, and T87 among the most likely residues in the closest interhelical contacts. Other candidates (L75, I91) are spaced at 4 residue intervals, which is consistent with the identification of a helical face. G79, G83, and T87 have previously been identified as experimentally crucial interfacial contacts (189). The analysis procedure I have employed therefore produces computational predictions of dimer interface residues that are consistent with experiment for GpA, and this provides confidence for the application of this method to the FGFR3 TM domain.

Three residues stand out from the equivalent aggregate analysis over all trajectories for each of the three FGFR3 conditions—G370, A374, R397 (Figure 5.20 on

page 153). Other prominent close contact residues are spaced at 3-4 residue intervals, which is consistent with the identification of a specific helical face of FGFR3. It is noteworthy that G380/R380 (the site of the achondroplasia mutation) does not feature prominently as a close contact residue, nor does A391 (mutation to E results in Crouzon syndrome) (190). Does it make sense that residues mutated in disease are not located directly at the dimer interface? Strikingly, G370 (one of the most prominent contacts) is mutated to C in type 1 thanatophoric dysplasia, which has a more severe phenotype than achondroplasia and is normally neonatal lethal (191). It is possible that the severity of the phenotype correlates with the proximity of the mutated residue to the dimer interface. Furthermore, G370 was recently localized to the FGFR3 dimer interface by site-specific infrared dichroism (192). The propensity for disulfide formation in FGFR3 follows the trend Cys370 > Cys371 > Cys375 (193), which is also consistent with an interfacial position for residue 370. R397, another of the prominent interfacial contacts, is part of the C-terminal CRLR tetrapeptide, which can be removed to increase the dimerization affinity of FGFR3 to match that of GpA (192).

5.4.6 Identification Of Dimer Interfaces

The experimental and clinical support for the proposed FGFR3 dimer interface residues is encouraging. Despite identification of a small set of candidate interfacial residues in the FGFR3 dimers, it is not clear if the broad distribution of allowable helix crossing angles (section 5.4.3 on page 114) results from sampling of different dimer interfaces. To test the possibility that more than one dimer interface can be sampled in the FGFR3 dimer, I employed a simplified reference frame. For all three FGFR3 conditions, a reference structure corresponding to the first helix in the first frame of the first WT FGFR3 simulation was used. The first helix in all frames of all FGFR3 replicate simulations was rmsd-fixed to match the configuration of that reference structure. Applying the same coordinate transformation to the second helix

allows for the assessment of the relative position of helix 2 while helix 1 is fixed in a single configuration. The geometric center of helix 2 was tracked in this adjusted coordinate system to test for the presence of multiple dimer interfaces. This is similar to a strategy previously employed to study the GpA dimer interface (184). I have written python MDAnalysis-dependent functions for calculating the positional probability of helix 2 in the rmsd-fixed reference frame of helix 1 for individual GpA and FGFR3 trajectories (fixed_helix_thermal()) and for results merged across all replicates for a given FGFR3 condition (fixed_helix_thermal_merged()). While the former function contains its own data-binning routine for outputting the helix 2 positional probabilities, the latter function is called prior to a separate binning routine (thermal_bins()). All three functions are stored in the python library dimer_geometric_tools.py D.17 on page 356, and controlled from the head script analyze_FGFR3_dimer_simulations.py D.15 on page 338.

The result for the first control replicate GpA trajectory (representative of the other GpA replicates) is shown in Figure 5.21 on page 154. The positional probability of helix 2 is consistent with a primary dimer interface at the 'bottom right' of helix 1 and a secondary interface at the 'top right.' Since ten replicate simulations were performed for each of the FGFR3 dimer conditions (Table 5.1 on page 189), the aggregate helix 2 positional probability results across all replicates were calculated for each condition (Figure 5.22 on page 155). All three FGFR3 constructs have a primary dimer interface at the 'bottom left' of helix 1. While the WT has no major dimer interaction at the 'bottom right' of helix 1, there is a progressively greater likelihood of helix 2 interacting with helix 1 at the 'bottom right' when looking at the WT \rightarrow heterodimer \rightarrow mutant homodimer results. Thus, a secondary dimer interface progressively appears in the FGFR3 heterodimer and mutant homodimer simulation conditions.

5.4.7 Dimer Interface Transitions

There is evidence for primary and secondary dimer interfaces for FGFR3 (and GpA) dimers (section 5.4.6 on page 118). It is not immediately clear how the helices transition between the interfaces. One possibility is a continuous sampling of the two interfaces with a bias toward the primary interface. However, a less frequent interface transition scheme is also possible—there may be discrete periods of the simulation at each of the interfaces with a single or a few transition points. To distinguish between these transition schemes I employed a simplified reference frame with rmsd-fixing of helix 1, as outlined in section 5.4.6 (page 118). However, instead of calculating the positional probability of helix 2, I simply tracked its geometric center during the trajectory by using a third plotting dimension—the frame (simulation time). The MDAnalysis-dependent python function frame_abstracted_relative_position() in the dimer_geometric_tools.py module D.17 (page 356) was used to parse individual GpA and FGFR3 trajectories in the described fashion.

An example of the result of this analysis on a representative GpA simulation trajectory is shown in Figure 5.23 on page 156. The stable helix 2 position at the 'bottom right' of helix 1 is apparent, with only one substantial excursion to the secondary dimer interface late in the simulation. The excursion is clearly discrete—there is a single point of exit from the primary interface and a single point of return to the primary interface. The other GpA replicates exhibit similar discrete (rather than continuous sampling) transitions between the primary and secondary dimer interfaces.

The FGFR3 mutant homodimer replicate 4 simulation is an interesting target for this analysis because there is a roughly equivalent positional probability for helix 2 at the primary and secondary dimer interfaces (Figure 5.24 on page 157). Tracking the geometric center of helix 2, it is apparent that helix 2 starts the simulation at the secondary interface ('bottom right' of helix 1), and exhibits a very large amplitude of motion at this interface (Figure 5.25 on page 158). However, roughly half way

through the trajectory there is a transition to the primary interface ('bottom left' of helix 1) where the position of helix 2 is much more stable. This is a representative result of discrete interface transitions in the mutant homodimer FGFR3 condition.

5.4.8 Identification Of Representative Dimer Interface Structures And Contacts

The FGFR3 mutant homodimer (and to some extent the heterodimer) has both primary and secondary dimer interfaces which are occupied for discrete periods of the simulations (section 5.4.7 on page 120). It would be informative to pull out a representative structure for each of the interfaces or to determine which interfacial residue contacts predominate each of the configurations. In principle, it should be possible to extract structures from simulation frames chosen manually from the plots in section 5.4.7. However, the 3D perspective view of these plots (*i.e.*, Figure 5.25 on page 158) makes it cumbersome to extract appropriate frames to represent a particular interface.

One way to simplify the plots is to remove one of the dimensions. It is crucial to track the simulation time or frame number because we need to know the exact frame that corresponds to a coordinate (interface position) for helix 2 in the reference frame of rmsd-fixed helix 1. However, instead of tracking both x and y coordinate positions it is possible to convert the Cartesian coordinates to a single polar angle θ (in the two-dimensional polar coordinate system).

An example plot of θ and Ω (helix crossing angle) tracked as a function of simulation frame number is shown in Figure 5.26 on page 159 for the first replicate GpA simulation. The excursions of helix 2 to the secondary dimer interface are discrete but brief for GpA, and there is apparently no substantial change in Ω for GpA dimer interface transitions. This is not surprising given the strong preference of GpA dimers for right-handed helix crossing angles (section 5.4.3 on page 114). In contrast, there appears to be a change in Ω as the FGFR3 dimer transitions from the secondary

to the primary interface in the fourth replicate mutant homodimer simulation (Figure 5.27 on page 160). Initially, at the secondary interface, Ω is primarily negative (right-handed) and then transitions to a bimodal distribution about 0 as the dimer assumes the primary configuration. It is also notable that there is a much larger amplitude of motion for FGFR3 helix 2 at the secondary interface when compared with its relatively stable position at the primary interface, consistent with observations in section 5.4.7 (page 120).

The demonstrated procedure for selection of representative dimer interface structures (simulation frames) from the helix 2 polar angle in the rmsd-fixed frame of helix 1 is 'manual,' and may not be the ideal way to address the selection of a representative structure across the population of all simulation replicate frames. To address the quality of the manually selected representative frames I have highlighted them in the context of a direct correlation between helix 2 polar θ and Ω for all frames in a given FGFR3 simulation (Figure 5.28 on page 160). The calculations were performed using the correlate_helixcrossing_polar_theta() function in the dimer_geometric_tools.py module D.17 (page 356). Even in the simple case of the FGFR3 WT there is an obvious complication with the manual selection procedure the selection of a single representative structure will always, by chance, pull out a leftor right-handed helix crossing configuration even though both are possible at the WT (primary) interface. The same problem crops up for selection of the representative primary interface in the FGFR3 heterodimer and mutant homodimer, and is potentiated by the comparison with the secondary interface in these conditions. Thus, while it is apparent that the FGFR3 secondary dimer interface represents mostly righthanded helix crossing configurations, the bimodal helix crossing angle distribution at the primary interface means that comparison of the representative primary and secondary interfaces would depend on the stochastic selection of a left- or right-handed dimer from the primary interface.

With the above limitations in mind, the FGFR3 dimer structures corresponding to the representative frames highlighted in Figure 5.28 (page 160) are shown in Figure 5.29 on page 161. By inspection, the FGFR3 secondary dimer interfaces are similar when compared between heterodimer and mutant constructs while there appear to be more substantial differences between representative primary interface structures. This is consistent with the wider variety of helix crossing angles at the primary interface and the stochastic component to manual structure selection described above.

The closest contacts by residue for each helix in the representative constructs were calculated using the closest_approach_representative() function in the dimer_geometric_tools.py module (D.17 on page 356). Plots of the reciprocal (for visualization purposes) closest contact distances for each residue in the constructs are shown in Figure 5.30 on page 162. The WT 'secondary' interface refers to a 'top left' position for helix 2 in Figure 5.22 on page 155, and is distinct from the secondary dimer interface which develops in the heterodimer and mutant. Nonetheless, there are some noticeable differences in the WT contacts between the two interfaces and R397 in particular has a much closer contact in the primary interface. The heterodimer interfaces differ strikingly at the N-terminus (including G370 and A374) while R397 and other C-terminal residue contacts are more similar. Less striking differences are observed between the interface contacts for the mutant homodimer, consistent with selection of representative constructs that have the same helix crossing angle (compare heterodimer and mutant selections in Figure 5.28 on page 160).

5.4.9 Population-Based Dimer Interface Classification

The previous section (5.4.8 on page 121) highlights the limitations with selection of single representative dimer interface structures for FGFR3. The next step is to use a clustering or population-based approach to classify groups of frames falling into representative categories to avoid the stochastic problems with selection of single

representative frames. GROMACS tools has a built-in clustering utility, g_cluster, which served as the starting point for this analysis. I used the g_cluster single linkage and gromos methods for generating the major clusters in each of the replicate simulations. Single linkage will add a frame to a cluster as long as it is within a specified rmsd-cutoff of any frame within that cluster, while the gromos algorithm is more sophisticated, using a neighbour clustering cut-off technique as described in (194). Plots correlating the % of trajectory structures in the major cluster with the rmsd of the major cluster are shown for the single linkage (Figure 5.31 on page 163) and gromos (Figure 5.32 on page 164) algorithms, as well as a direct comparison of the two (Figure 5.33 on page 165), using a 0.4 nm rmsd cutoff. The gromos algorithm is clearly more stringent—producing major clusters with lower rmsd values and therefore lower % frame incorporation into the major cluster. It is also notable that GpA trajectories consistently have less variation (lower rmsd), despite a similar or higher % incorporation, within their major clusters compared with FGFR3. This is consistent with the formation of a stronger dimer by GpA (188).

While the above GROMACS-based clustering methods produce sensible differences between GpA and FGFR3 trajectories, it would be preferable to incorporate the polar angle (θ of helix 2 in the reference frame of rmsd-fixed helix 1) into the population-based clustering method because θ (a change in position of helix 2) is the basis of the dimer interface classification. The basic idea is to select a population of frames within a certain set of θ bounds rather than a single frame within the interface boundary and then analyze the members of each interface population as a whole. One of the challenges for automated classification of simulation frames within populations of interfaces are the large-amplitude and short-lived excursions of FGFR3 away from the secondary interface and occasionally the primary interface. I do not want every brief excursion back and forth between primary and secondary interfaces to be treated as a stable transition from one interface to the other. Instead, I smoothed

the data for each FGFR3 replicate trajectory using a weighted moving average and a 'spike-filter.' Specifically, the simple_moving_average_polar_theta() function in the dimer_geometric_tools.py module D.17 (page 356) performs a few analysis steps: 1) For each replicate simulation, select only frames θ values that correspond to dimer configurations (frames after helix-helix closest $C_{\alpha} < 6$ Å). 2) Subject each data point to a 'spike filter:' if the current θ differs by more than 1.0 radians from the average θ values of both the 20 preceding and 20 following frames, its instantaneous θ is replaced by the average of the previous 10 data points. It is important to check both preceding and following θ trends because if θ only deviates substantially on one side of the current data point, this may reflect a stable transition between interfaces that is not an intended target for attenuation of a sudden change in θ . Note that using a large window for the weighted average instead of this kind of spike filter is also not desirable because it will produce a 'lag' in the data with the large window tail. 3) Use the standard python numpy.convolve() function to calculate a linear weighted moving average with a window size of 10 on the current spike-filtered data set. The simplification of the automated classification of frames to representative dimer interfaces as a result of the smoothed θ values is apparent with the attenuated fluctuations in the new data, as highlighted in Figure 5.34 on page 165.

Finally, the resulting data is parsed for the predominant interfacial residue contacts (as described in section 5.4.5 on page 116) at each of the interfaces, which are defined based on classification of the 'smoothed' polar θ values:

Primary interface (rad): $-3.0 < \theta < -1.5$

Secondary interface (rad): $-1.0 < \theta < 1.0$

Other interfaces (rad): remaining θ

The specific filtering and analysis implementation is available in the interface-

top_five_data() functions in the dimer_geometric_tools.py module D.17 (page 356). The predominant contacts, across all 30 FGFR3 dimer simulations (WT, heterodimer and mutant), for each of the FGFR3 dimer interfaces are shown in Figure 5.35 on page 166. There is a striking loss of contact symmetry at G370 (and to some extent R397) at the secondary interface compared with the primary interface. The other interface(s) exhibit a broad contact profile, which may simply reflect the fact that more than one discrete alternative interface exists outside the primary and secondary. Since G370 and R397 are candidates as important dimer contacts (section 5.4.5 on page 116), the present evidence is consistent with changes at critical dimer interface residues between primary and secondary interfaces and the existence of distinct configurations at each interface.

5.5 Analysis Of Lipid Bilayer In GpA And FGFR3 Simulations

The previous section (5.4 on page 111) concerns the analysis of the peptide components of the GpA and FGFR3 dimer trajectories. However, the peptides are not simulated in a vacuum and I will now focus on the analysis of the POPC lipid bilayer surrounding the TM segments in these simulations. One of the challenges with lipid bilayer analysis is the categorization of individual phospholipids to a particular leaflet. MDAnalysis propagates a network of connections between selected particles (*i.e.*, phosphates in lipid headgroups) using a cut-off distance which determines the leaflet groupings. This may cause problems if any lipid in one leaflet approaches a lipid in the other by the cut-off distance because the network of connections could then propagate through both leaflets and their separate definitions would be lost (Figure 5.36 on page 167). To simplify the tracking of lipid leaflets, I defined the leaflets in the first frame of each simulation and assigned each phosphate particle permanently

to a specific leaflet. In this manner, if a phospholipid moves closer to the center of the bilayer it will not abrogate the leaflet definitions because each phosphate is assigned permanently to one leaflet from the first frame (there is no propagation step in each frame).

5.5.1 Testing For Phospholipid 'Flip-Flop'

The method for leaflet selection outlined above handles bilayer pinching/narrowing more gracefully than a network propagation procedure in each frame. However, my method could produce misleading results if a phospholipid 'flip-flops' between leaflets and is treated as a member of the incorrect leaflet in a calculation. To test for lipid 'flip-flop' I wrote the flip_flop_tracker() function in the dimer_geometric_tools.py module (D.17 on page 356), which performs the following steps: 1) Selection of phosphate particles and their assignment to a particular leaflet, in the first frame of the simulation, by network propagation using the MDAnalysis built-in LeafletFinder function. The latter uses a networking cut-off optimized by the built-in optimize_cut-off function, which is called by the optimize_leaflet_selection_cutoff function in the dimer_geometric_tools.py module (D.17 on page 356) 2) With the absolute leaflet assignments complete, iterate through each frame of the simulation and track the largest and smallest Z (along bilayer normal) coordinates for each set of leaflet phosphates. 3) Calculate the average Z coordinate of all phosphates in the system for each frame to provide an estimate of the center of the bilayer.

This way, any replicate simulation can be tracked for phosphates which cross the center of the bilayer and enter the other leaflet, and I will simply discard those replicates with flip-flop activity from any leaflet-based analyses. One of the five GpA replicate simulations was discarded from bilayer analysis because of the inplane orientation assumed by one of the peptides during this simulation, while the other four did not show evidence of lipid flip-flop. A representative example from the fourth GpA replicate simulation is shown in Figure 5.37 on page 167. Interestingly, $\frac{2}{10}$

replicates in the FGFR3 WT condition exhibited lipid flip-flop between leaflets despite the same bilayer lipid (POPC). An example of a phosphate headgroup crossing the bilayer leaflet boundary, in the first replicate FGFR3 WT simulation, is shown in Figure 5.38 on page 168. None of the ten FGFR3 heterodimer replicates exhibited lipid flip-flop activity (*i.e.*, Figure 5.39 on page 169), nor did any of the ten FGFR3 mutant homodimer replicates (*i.e.*, Figure 5.40 on page 170).

The number of replicate simulations discarded because of lipid flip-flop is low, and the absolute assignment of lipid phosphates to a particular leaflet in the first frame of each simulation is therefore a robust method which can serve as the basis for subsequent bilayer parameter measurements.

5.5.2 Protein-Local And -Distal Bilayer Thickness

The previous section (5.5.1 on page 127) dealt with the limitations of my lipid bilayer leaflet selection procedure. With a robust method for leaflet selection established, the focus now switches to determination of the bilayer thickness near (local to) the TM monomers or dimers in comparison to the distal bilayer thickness (away from the TM segments). I have written the MDAnalysis-dependent python function analyze_leaflets() in the dimer_geometric_tools.py module D.17 (page 356) to perform a number of analysis steps on each of the replicate trajectories: 1) Assign top and bottom leaflet phosphate populations in the first frame as described above. 2) Select all phosphate particles in the top and bottom leaflets within a 16 Å shell of either TM segment. Also, select all phosphate particles in the top and bottom leaflets that are more than 16 Å away from both TM segments. 3) Calculate the difference between the Z coordinates of the centers of geometry for the respective phosphate particle populations selected in the previous step. These are estimates of bilayer thickness in protein-local and protein-distal regions of the system.

The interphosphate bilayer thickness tracked in protein-local and protein-distal regions is shown in a representative plot from the fourth GpA replicate results (Figure 5.41 on page 171). By inspection, there is at least a 4 Å thinning of the bilayer in protein-local versus -distal regions. While some protein-local bilayer thinning is also observed in the case of the FGFR3 WT, it is not nearly as substantial (*i.e.*, Figure 5.42 on page 172). Similar results were observed for the FGFR3 heterodimer replicates (*i.e.*, Figure 5.43 on page 173). One of the FGFR3 mutant homodimer replicates exhibited a dip in protein-local bilayer thickness roughly half way through the simulation (Figure 5.44 on page 173), but this was not observed for the majority of the mutant replicates.

The substantial POPC bilayer thinning observed near GpA may relate to hydrophobic mismatch with this 23 residue TM construct, while the moderate bilayer thinning in proximity to each of the FGFR3 constructs may be explained by the longer TM segments (33 residues). However, there is an additional concern with the bilayer thickness analysis presented in this section—are the 16 Å protein-local shells including a sufficient number of lipid phosphates? For example, if typically only two lipids are within the defined local shell of each peptide, there is an undesirably small sample size for the measurement. This is addressed in the next section.

5.5.3 Protein-Local And -Distal Lipid Shell Counts

The previous section (5.5.2 on page 128) includes analysis consistent with protein-local bilayer thinning in GpA and (to a lesser extent) in FGFR3 simulations. However, it is important to determine whether a sufficient number of protein-local lipid phosphates were captured in the defined 16 Å shells around the C_{α} particles of each TM segment. The count_lipids_in_local_shell() function in the dimer_geometric_tools.py module D.17 (page 356) was designed to count the number of phosphates in the top and bottom leaflets within 16 Å of either peptide in the dimer simulations as well as the total top and bottom leaflet phosphate counts for positions farther than 16 Å from either TM segment. Representative results for GpA are shown in Figure 5.45 on page 174. As expected, there are considerably more protein-distal than protein-

local lipids in each leaflet. It is noteworthy that the peptide dimerization event is clearly observable as a synchronized increase in the number of protein-distal lipids, and this is sensible because the protein-local shells overlap following dimerization. There are more lipids in the distal bottom leaflet relative to the distal top leaflet, but the reason for this is not clear and may simply relate to a partially random distribution of lipids during the POPC bilayer self-assembly process. A closer look at the peptide-local lipid shell counts reveals roughly 10 lipids per leaflet near a given GpA monomer (Figure 5.46 on page 174).

The FGFR3 WT also has a larger lipid population in the distal bottom leaflet versus the top (*i.e.*, Figure 5.47 on page 175). Again, there is a clear indicator of peptide dimerization as the distal leaflet lipid counts increase in unison, and the local lipid shell counts appear to synchronize at this time. The local shell count synchronization is especially apparent in the closer view (Figure 5.48 on page 175), which also reveals that 10-15 lipids are included per leaflet within 16 Å of a given FGFR3 monomer. In the pre-dimer state, one of the monomers fluctuates to some lower lipid shell counts.

The gap in distal leaflet lipid populations is much smaller in the FGFR3 heterodimer replicates (*i.e.*, Figure 5.49 on page 176), which is consistent with a stochastic component to leaflet population distribution during the bilayer self-assembly process. The ninth heterodimer replicate simulation tracked in Figure 5.49 again exhibits a synchronized increase in distal leaflet lipid populations coinciding with peptide dimerization (the extended time required for dimerization of this replicate is consistent with observations in section 5.4.1 on page 111). The local lipid shell counts also synchronize upon dimerization (Figure 5.50 on page 176), and 10-15 lipids are included per leaflet within 16 Å of a given FGFR3 monomer. Again, in the pre-dimer state, one of the monomers fluctuates to some lower lipid shell counts.

The behaviour of the FGFR3 mutant homodimer is very similar to the heterodimer

(i.e., Figure 5.51 on page 177 and Figure 5.52 on page 177). Overall, the 10-15 lipids per leaflet counted in the protein-local shells for GpA and FGFR3 replicates provide confidence that the 16 Å local shell definition is appropriate. A larger number of lipids (~ 90) was reported in a defined local shell around another simulated protein in the literature (195), but the rhomboid protease in question is substantially larger than the dimers reported here. With verification of the local lipid shell definition complete, the next section returns to the issue of tracking bilayer thickness, with an emphasis on the effect of dimerization on local bilayer thickness.

5.5.4 Overall Bilayer Thickness Analysis For GpA And FGFR3 Simulations

With added confidence in the protein-local and -distal lipid selection methodology (section 5.5.3 on page 129), and evidence for protein-local bilayer thinning in the CG simulations for GpA and FGFR3 (section 5.5.2 on page 128), it is desirable to analyze the data gathered across all replicate simulations for average bilayer thickness values before and after peptide dimerization. The function bilayer_thickness_average_results() in the dimer_geometric_tools.py module D.17 (page 356) performs a few tasks in this analysis: 1) Using the previously calculated closest interhelical C_{α} approach (per frame) during each of the trajectories (section 5.4.1 on page 111), determine the frame number at which dimerization occurs (defined as $d_{C_{\alpha}-C_{\alpha}} < 6$ Å). 2) Parse the previously calculated protein-local and -distal bilayer thickness results (section 5.5.2 on page 128) and split the protein-local data in to pre- and post-dimerization lists, combining results from each monomer. The protein-distal bilayer thickness values are not split on the dimerization frame. In total, this produces three separate lists for each of the GpA, FGFR3 WT, FGFR3 heterodimer, and FGFR3 mutant homodimer conditions.

Finally, the bilayer_stats() function in the same module is called separately by the head script (analyze_FGFR3_dimer_simulations.py on page 338) to deter-

mine the average and standard deviation values for the global lists produced by bilayer_thickness_average_results(). The overall results for GpA and the FGFR3 conditions are summarized in Figure 5.53 on page 178. While it is clear that GpA and FGFR3 TM peptides cause local bilayer thinning, it is not clear within one standard deviation that any substantial local bilayer thinning occurs following dimerization. The latter observation may be consistent with a stable dimerization process because additional local bilayer thinning following dimerization may come with an entropic lipid rearrangement penalty.

5.5.5 DPPC: The Effect Of Phospholipid Type On FGFR3 Dimer Behaviour

In addition to the manually setup and executed dimer simulations summarized in Table 5.1 (page 189), I also submitted FGFR3 WT, heterodimer and mutant homodimer configurations to the high-throughput GROMACS-based SIDEKICK automated simulation program (196). SIDEKICK performs the setup and execution of a large number of coarse-grained replicate simulations while abstracting the details from the user, and performed a total of 98 FGFR3 WT replicates, 96 FGFR3 heterodimer replicates, and 92 FGFR3 mutant homodimer replicates, with each replicate consisting of a 0.5 μ s trajectory. One limitation of this high-throughput framework is that only DPPC may be used as the bilayer lipid (because it has been sufficiently well characterized for coarse-grained membrane-based simulations to be used in an automated context). I've used POPC for the manually executed simulations for consistency with experimental FGFR3 studies (11, 173). However, I have performed a preliminary analysis on the SIDEKICK results to probe the effect of DPPC versus POPC on FGFR3 dimer behaviour.

5.5.5.1 FGFR3 Dimer Stability In DPPC

All of the GpA and FGFR3 constructs I have analyzed from CG simulations in POPC bilayers have formed stable dimers which do not dissociate once formed (see section 5.4.1 on page 111). However, WT and mutant GpA TM constructs have been observed to dissociate in DPPC-based CG simulations (184). In contrast, more recent DPPC-based CG simulations of WT and mutant GpA TM peptides do not exhibit any dissociation behaviour (185), and the authors suggest the discrepency with previous work is related to the more thoroughly calibrated MARTINI force field they employ.

FGFR3 WT, heterodimer, and mutant homodimer constructs all exhibited dissociation behaviour in DPPC bilayer-based SIDEKICK CG simulations (Figure 5.54 on page 179). The large number of replicate simulations were parsed by the closest_cont-acts_efficient_SIDEKICK() function in the dimer_geometric_tools.py module (page 356), which was controlled from a head script designed for the high-throughput data organization (analyze_sidekick_FGFR3_dimer_simulations.py). The reduced dimerization propensity is probably not the expected consequence of switching to a more saturated phospholipid, but given the variety of reported results within DPPC alone, it is not surprising that different phospholipids encourage varied dimerization behaviour.

5.5.5.2 FGFR3 Dimerization Interface In DPPC

In section 5.4.6 (page 118) I described a method for assessing the number of dimer interfaces explored by helix 2 when helix 1 is rmsd-fixed to a reference frame. For FGFR3, this reference frame is the configuration of helix 1 in the first frame of the first WT replicate simulation. Using the same (POPC-based) reference configuration for DPPC simulations, I have once again tracked the positional probability of helix 2, in this case to provide a direct comparison of the DPPC and POPC dimer interfaces for the three FGFR3 conditions. It is especially useful that the sample sizes are comparable between the SIDEKICK (DPPC) and manual (POPC) data sets. There were 10 replicates $\times 5 \frac{\mu_S}{replicate} = 50 \,\mu\text{s}$ total for each of the manual FGFR3 conditions. The 98 WT, 96 heterodimer, and 92 mutant FGFR3 0.5 μ s replicates accumulate 49,

48, and 46 μs of total simulation time, respectively. Although each FGFR3 condition can be compared over roughly 50 μs of total simulation time, it is noteworthy that the much larger number of SIDEKICK replicates results in more simulation restarts, and therefore less time spent in the dimer configuration.

The fixed_helix_thermal_merged_SIDEKICK() and thermal_bins_SIDEKICK() functions in the dimer_geometric_tools.py module (page 356) were called in sequence by the head script (analyze_sidekick_FGFR3_dimer_simulations.py) to produce the probability map for the position of helix 2 in the reference frame of rmsd-fixed helix 1. The comparison between FGFR3 dimer interfaces in POPC and DPPC is shown in Figure 5.55 on page 180. Clearly, the primary dimer interface is still located at the 'bottom left,' which is encouraging for validation as a stable interface. However, the secondary dimer interface is located at the 'top right' in DPPC rather than the 'bottom right' observed for POPC. Curiously, the secondary dimer interface in DPPC is more prominent in the WT than the heterodimer, and then reappears in the mutant homodimer.

5.6 FGFR3 Monomer Simulations

I performed a set of FGFR3 monomer simulations in POPC bilayers (see Table 5.2 on page 190) to control for the individual behaviour of the FGFR3 WT and G380R mutant TM peptides used in the dimer simulations. I wrote the preliminary analysis functions for the monomer replicates in the monomer_geometric_tools.py module (page 460), which is controlled by the head script analyze_FGFR3_monomer_simulations.py (page 457).

5.6.1 Helix Tilt Angle

I wrote the helix_tilt_vs_bilayer_normal() function in the monomer_geometric_tools.py module (page 460) to track the tilt of the FGFR3 WT or mutant helices with respect to the bilayer normal in each of the replicate monomer trajectories. The

helical axis was defined as the first eigenvector of the C_{α} backbone and the bilayer normal was defined as the third eigenvector of the POPC phosphate headgroups. Both the WT (*i.e.*, Figure 5.56 on page 181) and mutant (*i.e.*, Figure 5.57 on page 182) monomers exhibited frequent fluctuations in helical tilt angles, mostly between 0° and 30°. This is a wider range of fluctuation than predicted by oriented circular dichroism and neutron diffraction of FGFR3 peptides in POPC bilayers (0° to 20°) (11).

5.6.2 FGFR3 SIDEKICK Monomer Simulations

In addition to the manually executed monomer replicates, the SIDEKICK high-throughput simulation framework (described in section 5.5.5 on page 132) was used to conduct 100 replicate simulations (of 0.1 μ s duration) for each of the WT and G380R FGFR3 TM constructs in POPC bilayers. In contrast to the case for dimer simulations, SIDEKICK allows for the use of POPC and automatically generates a gallery of analysis plots. The peptide bilayer burial depth distributions are plotted for the WT (Figure 5.58 on page 183) and mutant (Figure 5.59 on page 184), and it is clear that the center of the mutant peptide is displaced upward from the center of the bilayer relative to the WT (by at least 1 Å). This is consistent with the introduction of the R residue in the mutant TM segment and the movement of this residue away from the hydrophobic core of the bilayer. Experimental results for FGFR3 peptide constructs in POPC bilayers were consistent with a 5 Å upward (N-terminal) displacement of the R380 residue relative to G380 in the WT (11).

SIDEKICK also automatically produced plots for the FGFR3 WT (Figure 5.60 on page 185) and mutant (Figure 5.61 on page 186) helix tilt angle distributions over all the replicate simulations. Both WT and mutant constructs exhibited similar helix tilt angle distribution modes ~38°. This angle is larger than the upper value of the range predicted by experimental constraints from oriented circular dichroism and neutron diffraction of FGFR3 peptides in POPC bilayers (0° to 20°) (11).

The final analysis completed by SIDEKICK is the distribution of helix rotation

angles (about the helical axis itself). While the WT FGFR3 helix clearly assumes a single favoured rotation angle in the bilayer (Figure 5.62 on page 187), the G380R mutant exhibits a bimodal distribution of rotation angles with a roughly 180° rotation between the preferred configurations (Figure 5.63 on page 188). This added 'flexibility' may contribute to the secondary dimer interface behaviour observed in heterodimer and mutant homodimer (section 5.4.6 on page 118).

5.7 Summary And Conclusions

I started this chapter by describing the medical relevance of the G380R mutation in the TMD of FGFR3—in ~99% of cases it is the underlying cause of achondroplasia (163, 164, 165). CG-MD simulations of the FGFR3 WT homodimer, heterodimer, and mutant homodimer TMDs in lipid bilayers were analyzed in detail (section 5.4 on page 111). I demonstrated that the initial 55 Å separation between helices was sufficient to ensure that there was no bias in the dimer formation process and that the dimers did not dissociate once formed (section 5.4.1 on page 111). A substantial amount of stochastic wandering can occur in the bilayer prior to dimer formation (section 5.4.2 on page 113), and this is also consistent with unbiased dimer formation. FGFR3 dimer constructs all exhibited bimodal helix crossing angles (section 5.4.3 on page 114), but still moved together in the membrane after dimer formation (section 5.4.4 on page 115).

Having confirmed stable and unbiased dimer formation, I parsed the GpA and FGFR3 trajectories for the closest interfacial contacts in the dimers (section 5.4.5 on page 116). I first tested my analytical approach on the GpA constructs and the major contacts parsed from the simulations were consistent with experimental results. This provided confidence for the application of the analysis to FGFR3 and I identified at least three major interfacial residues—G370, A374, and R397. The latter residues were consistent with experimental and medical findings, and are suggested as candi-

date interfacial residues for FGFR3 dimers (for which no high-resolution structures are currently available). The three FGFR3 dimer constructs all have a consistent primary dimer interface, but also a secondary dimer interface that appears progressively in the heterodimer and is most prominent in the mutant homodimer (section 5.4.6 on page 118). I demonstrated that the FGFR3 helical positions are more stable at the primary interface than the secondary interface, and that there is a discrete time spent at each interface rather than a continuous sampling between the two interfaces (section 5.4.7 on page 120). Attempts to manually select representative dimer interface structures based on the polar θ of helix 2 in the rmsd-fixed reference frame of helix 1 were problematic because there is a stochastic component to the manual selection procedure that can select a representative primary interface structure that has either a left- or right-handed helix crossing angle (section 5.4.8 on page 121).

For a rigorous classification of FGFR3 dimer interfaces, I parsed across the frames of all trajectories to define *populations* of simulation frames that fall into the primary, secondary, and 'other' interfaces based on moving average (sliding window) polar θ and a filter for sudden short-lived fluctuations in the data (section 5.4.9 on page 123). The results were consistent with a loss of symmetry at G370 and R397 at the secondary interface compared with the primary interface. These interfacial differences are at important contacts (based on simulation and experiment) and are consistent with rotation of one helix relative to the other—a potential molecular mechanism for pathology. In support of this model, activation of ErbB2 occurs by a 120° rotation of the TMD monomers relative to each other after ligand binding to the RTK (197). In addition, the oncogenic V664E TMD mutation in Neu (rat homologue of ErbB2) prevents conformational switching between the active and inactive states (198). Thus, activating RTK TMD mutations may encourage or restrict the rotation of TMDs depending on the specific structure of the RTK.

In section 5.5 (page 126) I analyzed some properties of the POPC bilayer local

and distal to the FGFR3 TMD peptides. There was peptide-local bilayer thinning for GpA and (to a lesser extent) for FGFR3. Curiously, I found that all three FGFR3 dimer constructs dissociated in DPPC but not in POPC, and that the secondary dimer interface and its trends are different in DPPC (section 5.5.5 on page 132).

FGFR3 monomer simulations in POPC were consistent with increased vertical (N-terminal) displacement of the mutant monomer from the center of the bilayer (relative to FGFR3 WT) (section 5.6.2 on page 135), similar helix tilt angle distributions (relative to the bilayer normal), and the presence of two preferred rotation (about the helical axis) angles for the mutant constructs (and only a single preferred rotation angle for the WT). The latter observation may relate to the observed secondary dimer interface for dimer constructs involving G380R and may in particular explain the ability of this helix to rotate in the membrane between the two interfaces.

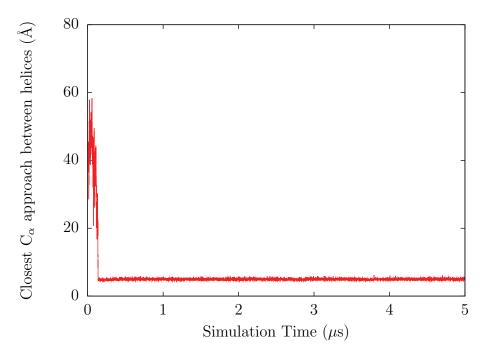


Figure 5.1: Tracking closest C_{α} helix-helix approach for the first GpA coarse-grained simulation.

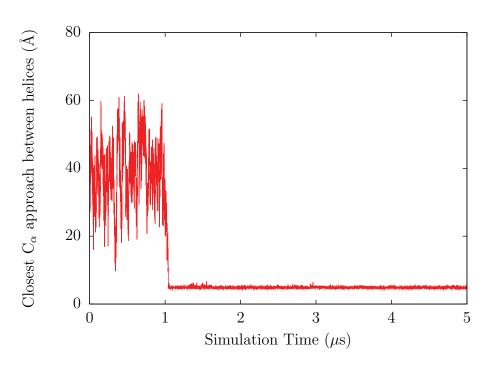


Figure 5.2: Tracking closest C_{α} helix-helix approach for the third WT FGFR3 coarse-grained simulation.

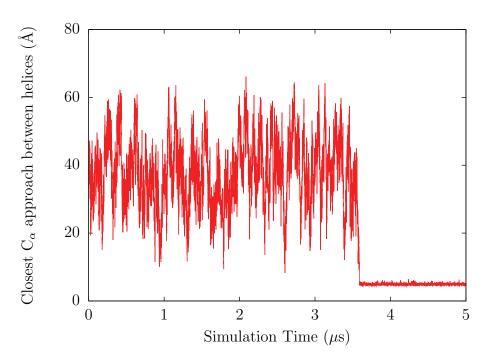


Figure 5.3: Tracking closest C_{α} helix-helix approach for the ninth FGFR3 heterodimer coarse-grained simulation.

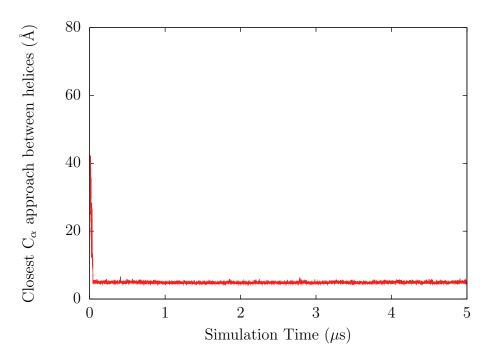


Figure 5.4: Tracking closest C_{α} helix-helix approach for the second FGFR3 mutant homodimer coarse-grained simulation.

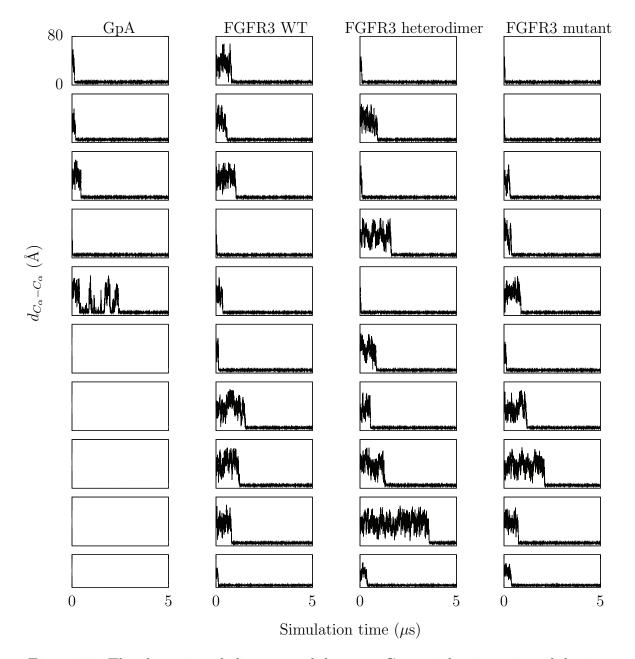


Figure 5.5: The closest interhelix approach between C_{α} particles is monitored during the 5 μs coarse-grained simulations. Five replicates were conducted for GpA, while ten replicates were completed for each of FGFR3 wild-type, G380R heterodimer, and G380R mutant homodimer conditions.

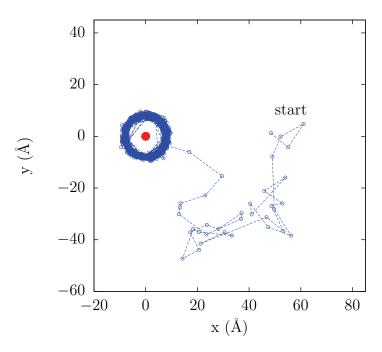


Figure 5.6: Relative motion of the center of geometry of helix 2 (blue) in the reference frame of the center of geometry of helix 1 (red) during the first wild-type homodimer GpA coarse-grained simulation (every 10th frame).

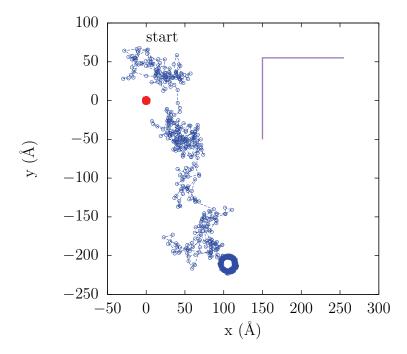


Figure 5.7: Relative motion of the center of geometry of helix 2 (*blue*) in the reference frame of the center of geometry of helix 1 (*red*) during the seventh FGFR3 WT homodimer replicate coarse-grained simulation (every 10th frame). The approximate size of the simulation box in the x-y plane is shown in *purple*.

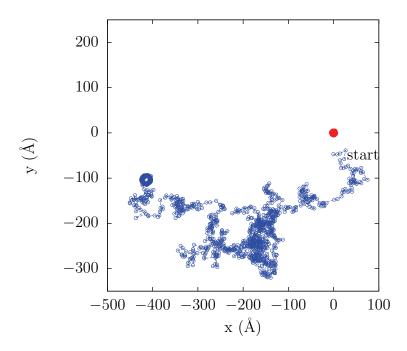


Figure 5.8: Relative motion of the center of geometry of helix 2 (blue) in the reference frame of the center of geometry of helix 1 (red) during the ninth FGFR3 heterodimer replicate coarse-grained simulation (every $10^{\rm th}$ frame).

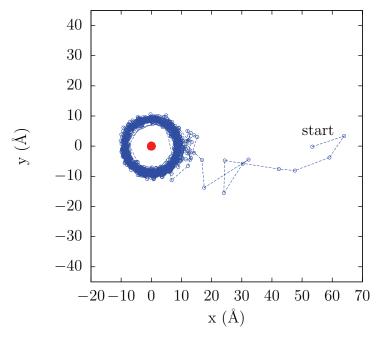


Figure 5.9: Relative motion of the center of geometry of helix 2 (*blue*) in the reference frame of the center of geometry of helix 1 (*red*) during the second FGFR3 mutant homodimer replicate coarse-grained simulation (every 10th frame).

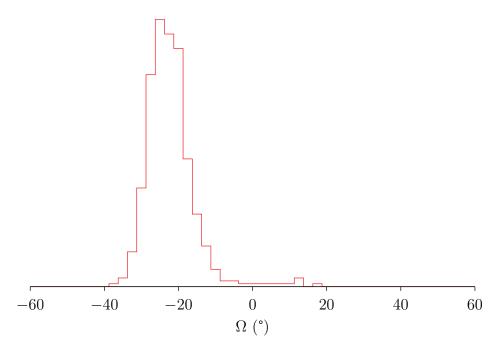


Figure 5.10: Helix crossing angle (Ω) distribution for the first replicate of the GpA WT homodimer construct. Every 25th frame of the trajectory was parsed and included in the histogram only if the helices were dimerized. The area under the curve sums to unity.

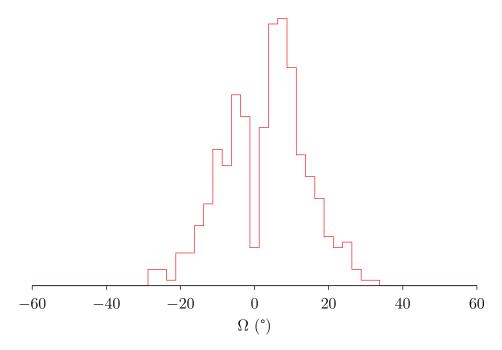


Figure 5.11: Helix crossing angle (Ω) distribution for the first replicate of the FGFR3 WT homodimer construct. Every $25^{\rm th}$ frame of the trajectory was parsed and included in the histogram only if the helices were dimerized. The area under the curve sums to unity.

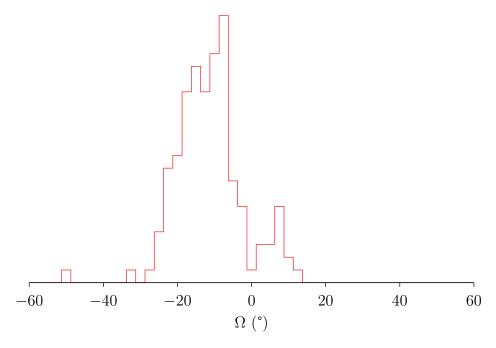


Figure 5.12: Helix crossing angle (Ω) distribution for the ninth replicate of the FGFR3 heterodimer construct. Every $25^{\rm th}$ frame of the trajectory was parsed and included in the histogram only if the helices were dimerized. The area under the curve sums to unity.

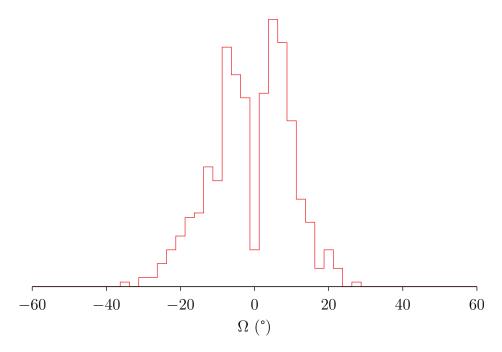


Figure 5.13: Helix crossing angle (Ω) distribution for the first replicate of the FGFR3 mutant homodimer construct. Every 25th frame of the trajectory was parsed and included in the histogram only if the helices were dimerized. The area under the curve sums to unity.

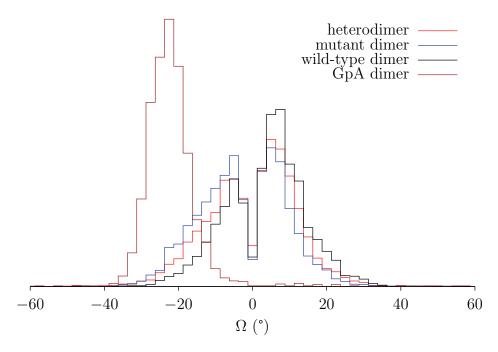


Figure 5.14: Helix crossing angle (Ω) distribution merged over all replicates of GpA and FGFR3 constructs. Every 25th frame of the constituent trajectories was parsed and included in the histograms only if the helices were dimerized. Helix crossing angles are shown for GpA (*brown*), FGFR3 wild-type (*black*), FGFR3 heterodimer (*red*), and FGFR3 mutant homodimer (*blue*). The area under each curve sums to unity.

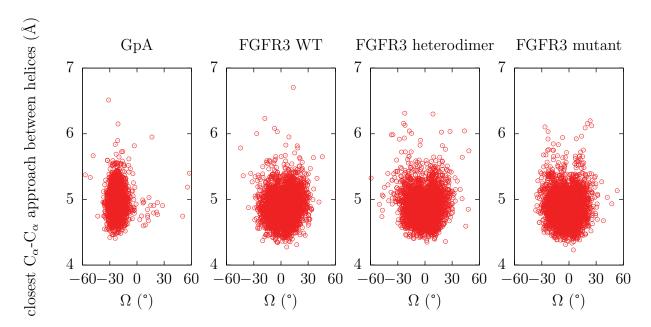


Figure 5.15: Correlating the closest C_{α} interhelical approach with helix crossing angle (Ω) for GpA (5 replicates), FGFR3 WT (10 replicates), FGFR3 heterodimer (10 replicates), and FGFR3 mutant homodimer (10 replicates) constructs. The plotted results are merged across all available replicate simulations as indicated.

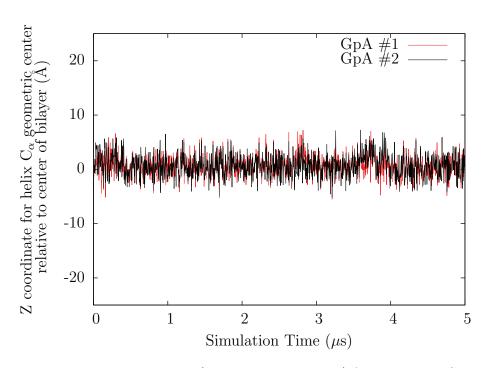


Figure 5.16: Tracking Z coordinate (along bilayer normal) for each helix C_{α} geometric center relative to center of bilayer during the first GpA replicate simulation. Every 10^{th} frame was parsed in a non-centered trajectory.

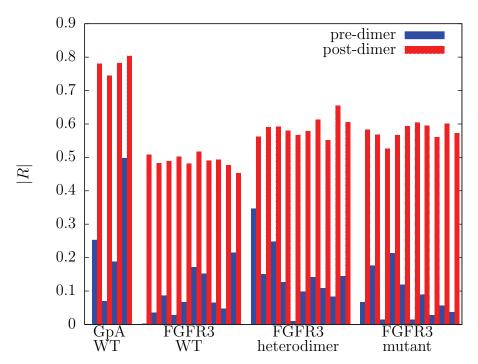


Figure 5.17: The absolute correlation coefficients (|R|) between the Z coordinates of the geometric centers of the GpA or FGFR3 helices in each replicate simulation before and after dimerization. The dimerization distance is defined as a closest interhelical C_{α} approach < 6 Å.

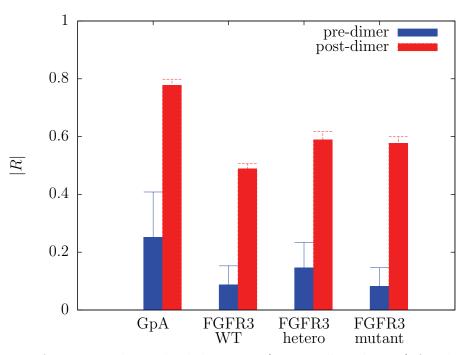


Figure 5.18: Average and standard deviation (across all replicates) for the absolute correlation coefficient (|R|) between helical geometric center Z coordinates for GpA and FGFR3 dimer simulation constructs before and after dimerization. The dimerization distance is defined as a closest interhelical C_{α} approach < 6 Å.

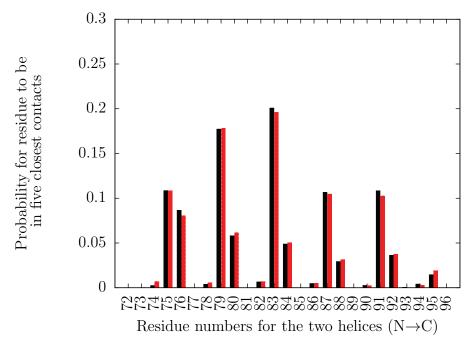


Figure 5.19: The normalized frequency of occurrence for a residue in the five closest contacts between helix 1 (black) and helix 2 (red) when the GpA helix-helix C_{α} separation is within 7 Å in the first replicate coarse-grained simulation.

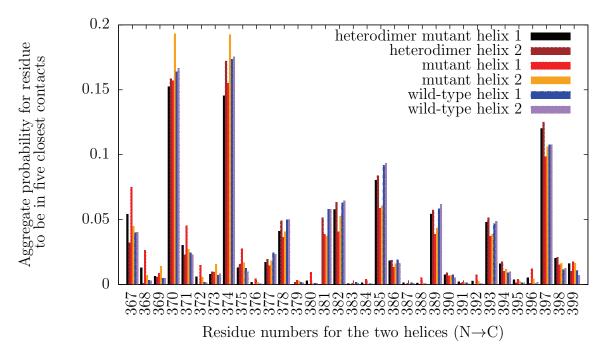


Figure 5.20: Normalized frequency of occurence for a residue in the five closest contacts between helices when interhelix C_{α} separation is within 7 Å over *all* the CG replicate simulations for each of FGFR3 wild-type (helix 1 *blue*, helix 2 *purple*), heterodimer (G380R helix 1 *black*, WT helix 2 *brown*), and mutant homodimer (helix 1 *red*, helix 2 *yellow*) conditions.

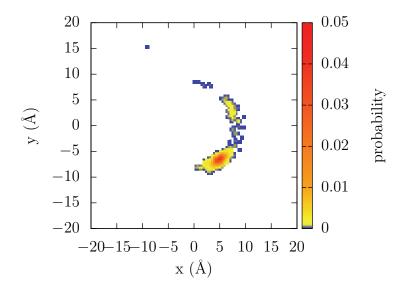


Figure 5.21: A contour plot of the positional probability of GpA helix 2 in the reference frame of rmsd-fixed helix 1 (centered at the origin) in the first GpA replicate simulation. The non-linear probability scale is indexed as $P = 0.0 \ white$, $P = 0.000001 \ blue$, $P = 0.001 \ yellow$, $P = 0.01 \ orange$, $P = 0.05 \ red$.

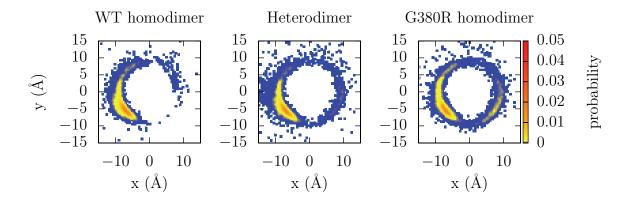


Figure 5.22: A contour plot of the positional probability of helix 2 in the reference frame of rmsd-fixed helix 1 (centered at the origin) calculated over all ten replicate trajectories for each of the FGFR3 dimer conditions. The non-linear probability scale is indexed as P=0.0 white, P=0.000001 blue, P=0.001 yellow, P=0.01 orange, P=0.05 red.

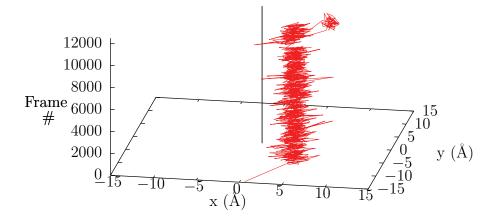


Figure 5.23: The coordinates of the geometric center of helix 2 (*red line*) are tracked in the reference frame of rmsd-fixed helix 1 (central *black line*) as the simulation progresses (with frame number along the Z-axis). This is the fourth replicate GpA CG simulation and is representative of results for other GpA replicates.

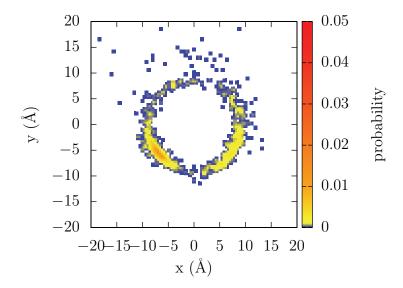


Figure 5.24: A contour plot of the positional probability of helix 2 in the reference frame of rmsd-fixed helix 1 (centered at the origin) in the fourth mutant homodimer FGFR3 simulation. The non-linear probability scale is indexed as P=0.0 white, P=0.000001 blue, P=0.001 yellow, P=0.01 orange, P=0.05 red.

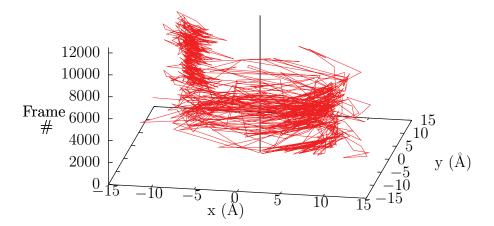


Figure 5.25: The coordinates of the geometric center of helix 2 (*red line*) are tracked in the reference frame of rmsd-fixed helix 1 (central *black line*) as the simulation progresses (with frame number along the Z-axis). This is the fourth replicate FGFR3 mutant homodimer CG simulation and is representative of results for other mutant replicates.

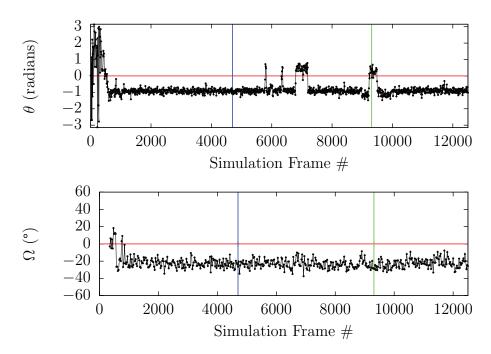


Figure 5.26: Polar angle of helix 2 (θ , top) in the rmsd-fixed frame of helix 1 and helix crossing angle (Ω , bottom) are tracked during the first GpA replicate simulation. Vertical lines are drawn in the respective plots to highlight frames that represent the primary (*blue*, frame 4701) and secondary (*green*, frame 9311) dimer interfaces based on their polar angles. The excursions to the secondary dimer interface are discrete but brief for GpA.

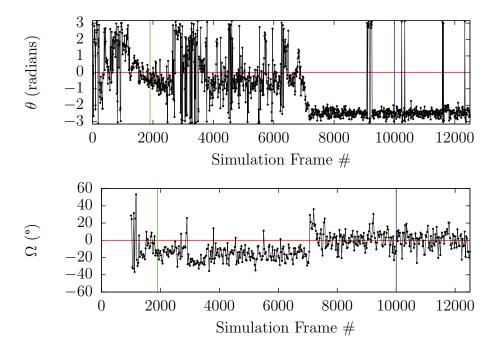


Figure 5.27: Polar angle of helix 2 (θ , top) in the rmsd-fixed frame of helix 1 and helix crossing angle (Ω , bottom) are tracked during the fourth FGFR3 mutant homodimer replicate simulation. Vertical lines are drawn in the respective plots to highlight frames that represent the primary (blue, frame 10001) and secondary (green, frame 1901) dimer interfaces based on their polar angles. There appears to be a transition in Ω that coincides with the dimer interface transition.

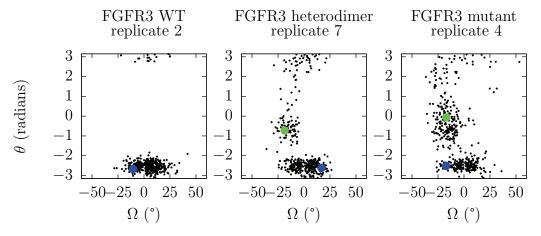


Figure 5.28: Direct correlation of polar angle (θ) and helix crossing angle (Ω) for the frames of representative FGFR3 simulations. The specific primary (blue) and secondary (green) representative dimer interface frames selected by the manual procedure are highlighted as appropriate.

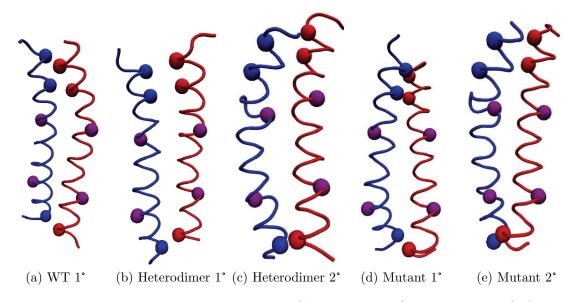


Figure 5.29: Representative coarse-grain C_{α} structures (N-terminus top) for the FGFR3 primary and secondary dimer interfaces. G370, A374, and R397 (which are among the predominant interfacial contacts in Figure 5.20 on page 153) are shown in van der Waals representation, and disease-target residues G/R380 and A391 are in *purple*. Similar secondary interface structures for heterodimer (c) and mutant (e) reflect right-handed crossing angles while differences between primary interface structures reflect (in part) variation in helix crossing angles (which have a bimodal distribution at the FGFR3 primary interface as highlighted in Figure 5.28 on page 160)

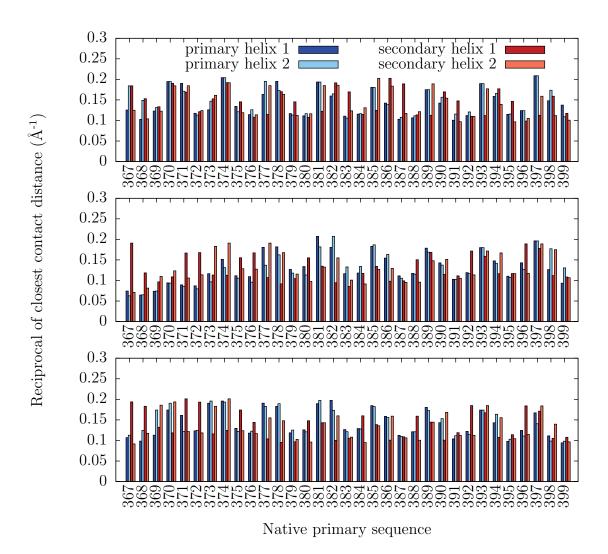


Figure 5.30: FGFR3 WT (top), heterodimer (middle), and mutant homodimer (bottom) reciprocal closest contact distances for each residue in each helix of a representative dimer interface structure.

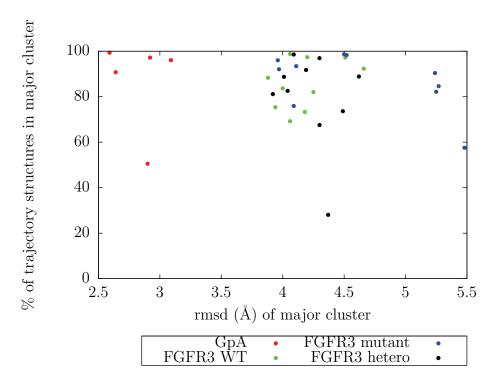


Figure 5.31: Comparing coarse-grained MD simulation trajectory structural clustering by the single linkage method (0.4 nm cutoff; every $10^{\rm th}$ frame parsed). The fifth replicate GpA simulation has a smaller % incorporation to the major cluster because one of the helices assumes an in-plane orientation during part of that simulation. An even smaller % incorporation is observed for the $9^{\rm th}$ replicate FGFR3 heterodimer simulation because of the nearly 4 μ s time required for dimerization.

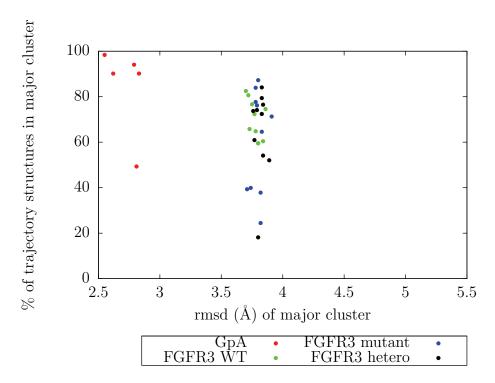


Figure 5.32: Comparing coarse-grained MD simulation trajectory structural clustering by the gromos algorithm (0.4 nm cutoff; every $10^{\rm th}$ frame parsed). The fifth replicate GpA simulation has a smaller % incorporation to the major cluster because one of the helices assumes an in-plane orientation during part of that simulation. An even smaller % incorporation is observed for the $9^{\rm th}$ replicate FGFR3 heterodimer simulation because of the nearly 4 μs time required for dimerization.

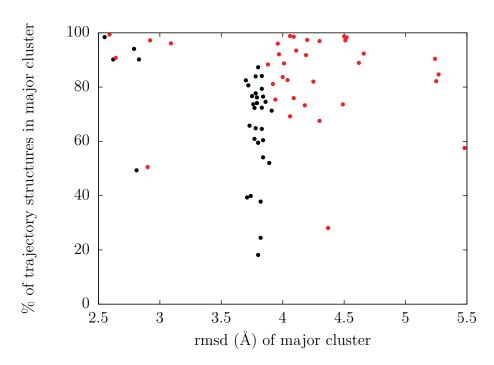


Figure 5.33: Comparing gromos (black) and single linkage (red) algorithms for clustering 35 dimer simulations involving FGFR3 or GpA (0.4 nm cutoff; every $10^{\rm th}$ frame).

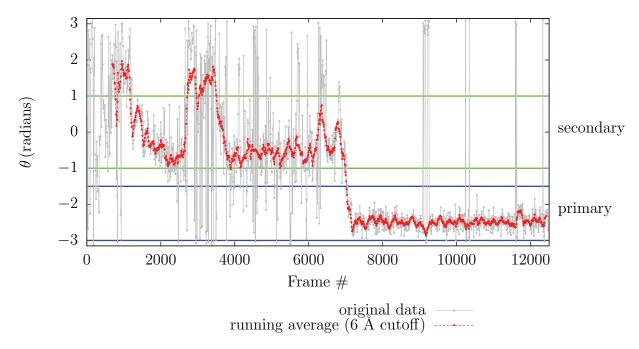


Figure 5.34: Using a weighted moving average and spike-filter to classify frames according to helix 2 COM θ in rmsd-fixed reference frame of helix 1 in the fourth replicate FGFR3 mutant simulation.

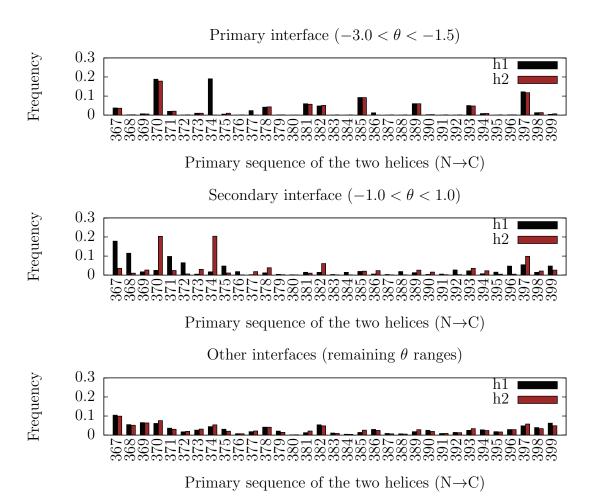


Figure 5.35: Normalized frequency of occurrence for a residue in the five closest contacts between helices when interhelix (C_{α}) separation is within 6 Å for primary, secondary and 'other' dimer interface populations from *all* 30 FGFR3 CG replicate simulations.

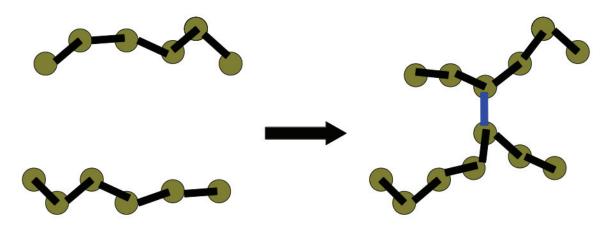


Figure 5.36: Using an MDAnalysis networkx-based utility, a set of phosphate particles (circles) are connected within a certain cut-off boundary to produce two clearly separated leaflets (left) or there is a failure to identify separate leaflets because of phosphate headgroups with an intermediate position (right). The latter is a concern because it may occur during a subset of frames in a simulation when a particular lipid particle is substantially perturbed away from the regular surface of the leaflet, causing failure of any measurement that depends on the identification of two separate leaflets.

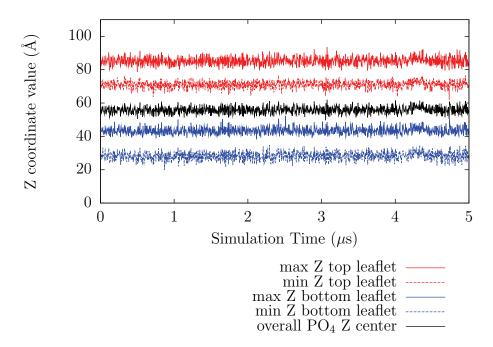


Figure 5.37: Checking for phospholipid 'flip-flop:' tracking POPC leaflet minimum and maximum phosphate Z coordinates for the fourth coarse-grained GpA simulation (every 10th frame). It is apparent that no phosphate particle crosses the approximate center boundary of the bilayer.

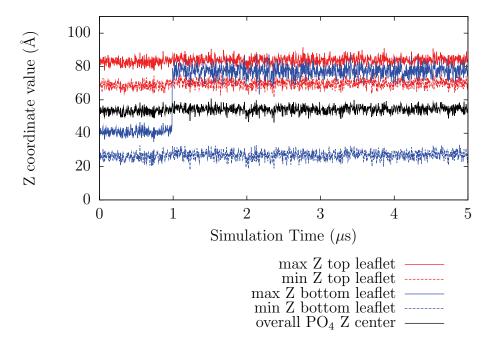


Figure 5.38: Checking for phospholipid 'flip-flop:' tracking POPC leaflet minimum and maximum phosphate Z coordinates for the first coarse-grained FGFR3 WT simulation (every 10th frame). It is apparent that a phosphate particle crosses the approximate center boundary of the bilayer.

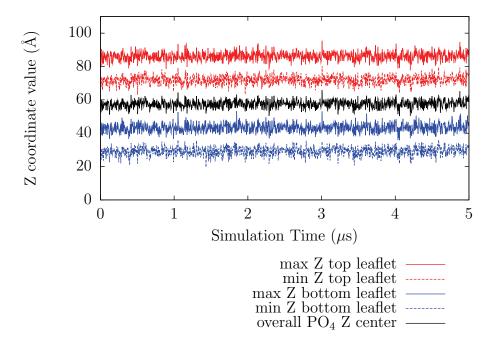


Figure 5.39: Checking for phospholipid 'flip-flop:' tracking POPC leaflet minimum and maximum phosphate Z coordinates for the tenth coarse-grained FGFR3 heterodimer simulation (every 10th frame). It is apparent that no phosphate particle crosses the approximate center boundary of the bilayer.

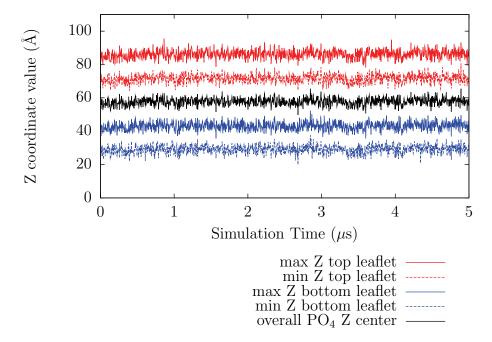


Figure 5.40: Checking for phospholipid 'flip-flop:' tracking POPC leaflet minimum and maximum phosphate Z coordinates for the tenth coarse-grained FGFR3 mutant homodimer simulation (every 10th frame). It is apparent that no phosphate particle crosses the approximate center boundary of the bilayer.

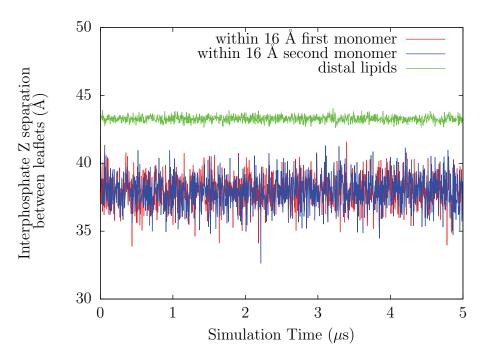


Figure 5.41: Comparing leaflet POPC interphosphate distance (bilayer thickness) for protein-local and -distal regions in the fourth coarse-grained GpA simulation (every $10^{\rm th}$ frame).

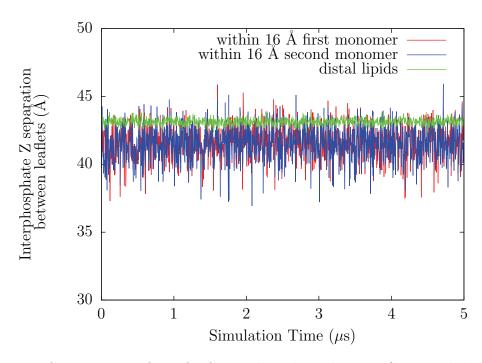


Figure 5.42: Comparing leaflet POPC interphosphate distance (bilayer thickness) for protein-local and -distal regions in the tenth coarse-grained FGFR3 WT simulation (every $10^{\rm th}$ frame).

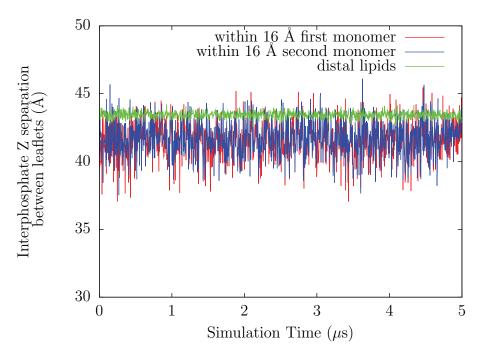


Figure 5.43: Comparing leaflet POPC interphosphate distance (bilayer thickness) for protein-local and -distal regions in the tenth coarse-grained FGFR3 heterodimer simulation (every 10th frame).

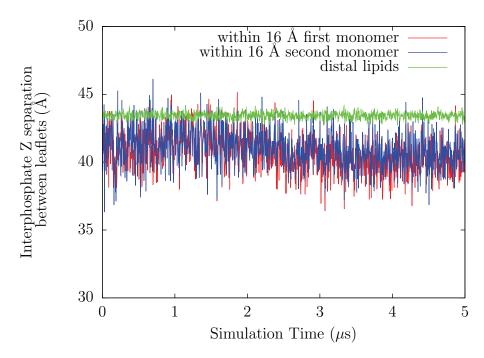


Figure 5.44: Comparing leaflet POPC interphosphate distance (bilayer thickness) for protein-local and -distal regions in the ninth coarse-grained FGFR3 mutant homodimer simulation (every 10th frame).

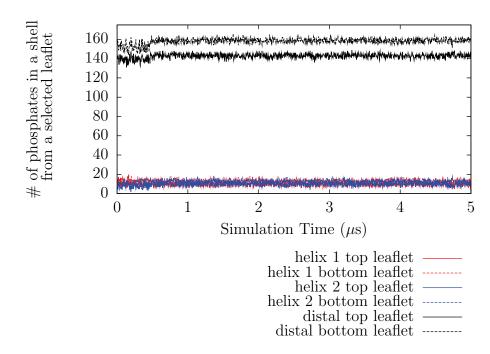


Figure 5.45: Tracking the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the third replicate GpA CG simulation (every 10th frame). The remaining (protein-distal) lipid phosphate counts are also tracked.

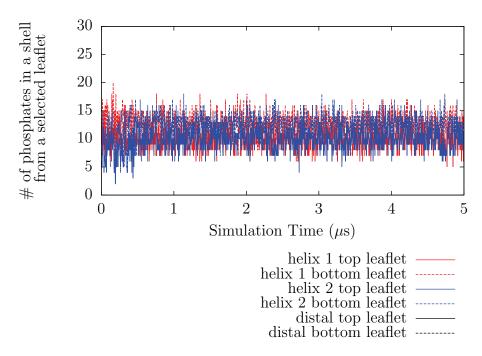


Figure 5.46: This is a zoom-in version of Figure 5.45 (page 174) focusing on the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the third replicate GpA CG simulation.

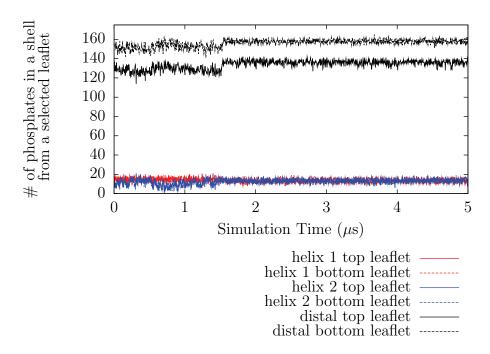


Figure 5.47: Tracking the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the seventh replicate FGFR3 WT CG simulation (every 10th frame). The remaining (protein-distal) lipid phosphate counts are also tracked.

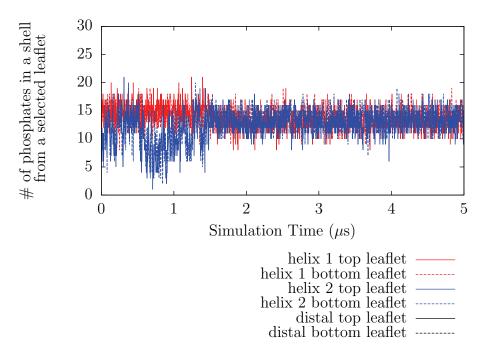


Figure 5.48: This is a zoom-in version of Figure 5.47 (page 175) focused on the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the seventh replicate FGFR3 WT CG simulation.

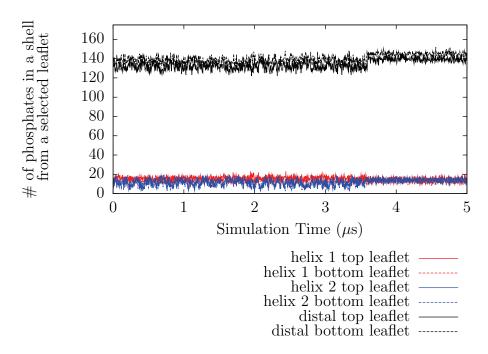


Figure 5.49: Tracking the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the ninth replicate FGFR3 heterodimer CG simulation (every 10th frame). The remaining (protein-distal) lipid phosphate counts are also tracked.

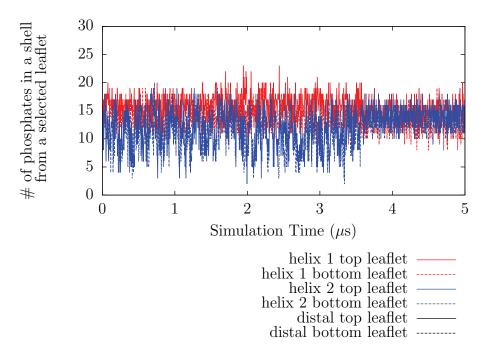


Figure 5.50: This is a zoom-in version of Figure 5.49 (page 176) focused on the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the ninth replicate FGFR3 heterodimer CG simulation.

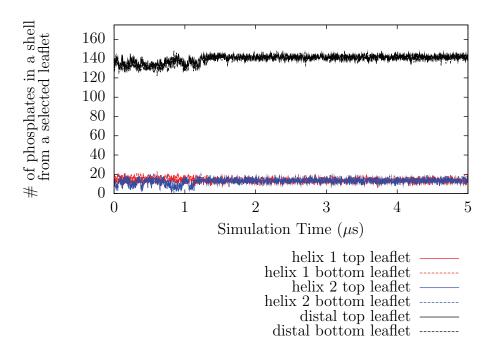


Figure 5.51: Tracking the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the seventh replicate FGFR3 mutant homodimer CG simulation (every 10th frame). The remaining (protein-distal) lipid phosphate counts are also tracked.

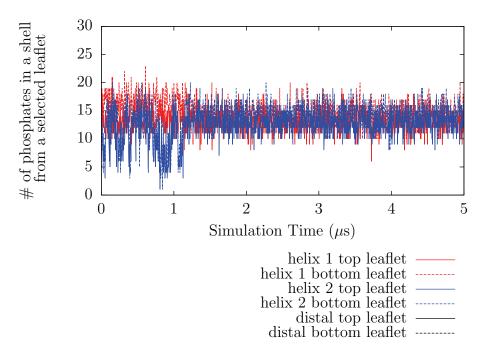


Figure 5.52: This is a zoom-in version of Figure 5.51 (page 177) focused on the number of lipid phosphates within 16 Å of either TM peptide (set of C_{α} particles) for each bilayer leaflet in the seventh replicate FGFR3 mutant homodimer CG simulation.

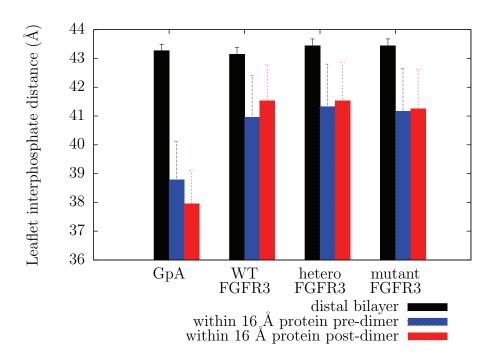


Figure 5.53: Average and standard deviation values for interphosphate bilayer thickness in GpA and FGFR3 simulation conditions. The protein-local thickness values are measured within 16 Å of the peptides and the pre- and post-dimerization divisions are based on a closest interhelical C_{α} approach of < 6 Å.

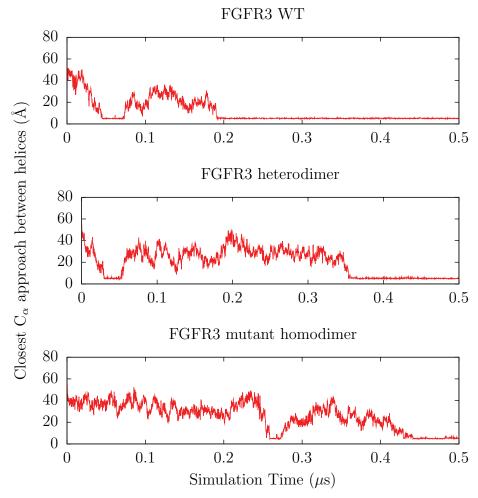


Figure 5.54: Tracking the closest C_{α} interhelical distance for representative SIDEKICK-based CG simulations in DPPC bilayers. Dimer dissociation events are conspicuous, in contrast to the results with POPC bilayers in section 5.4.1 (page 111).

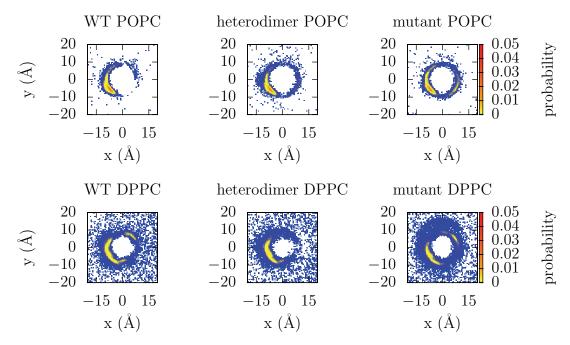


Figure 5.55: Probability map for the position of helix 2 in the rmsd-fixed reference frame of helix 1 compared for FGFR3 CG simulations in POPC (top) and DPPC (bottom). The reference structure for rmsd alignment is always the configuration of helix 1 in the first frame of the first WT POPC simulation. The non-linear probability scale is indexed as $P = 0.0 \ white$, $P = 0.000001 \ blue$, $P = 0.001 \ yellow$, $P = 0.01 \ orange$, $P = 0.05 \ red$.

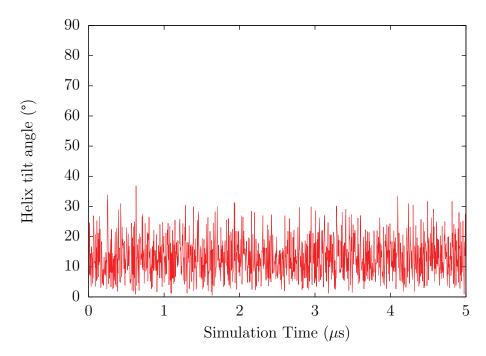


Figure 5.56: Representative plot tracking the helix tilt angle of the FGFR3 WT monomer (replicate 1) relative to the bilayer normal. The helix axis was defined as the first eigenvector of the C_{α} backbone and the bilayer normal was calculated as the third eigenvector of the POPC phosphate population.

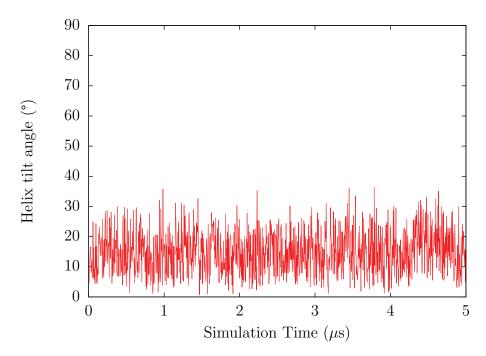


Figure 5.57: Representative plot tracking the helix tilt angle of the FGFR3 mutant monomer (replicate 1) relative to the bilayer normal. The helix axis was defined as the first eigenvector of the C_{α} backbone and the bilayer normal was calculated as the third eigenvector of the POPC phosphate population.

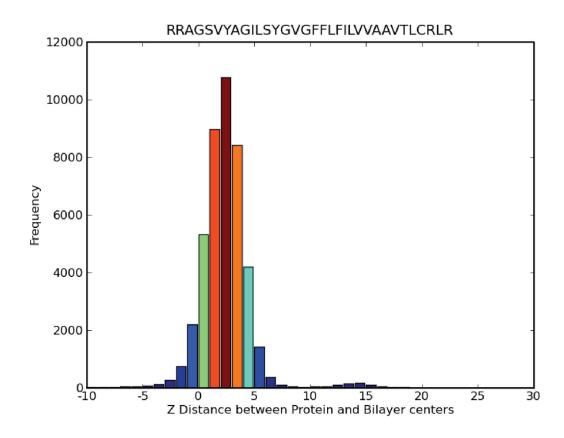


Figure 5.58: The Z separation (along POPC bilayer normal, in Å) between the center of the FGFR3 WT peptide and the center of the bilayer was recorded across 100 (0.1 μ s) replicate simulations and the frequency distribution is plotted here. The mode of the distribution is an upward (N-terminal) vertical displacement of ~ 3 Å.

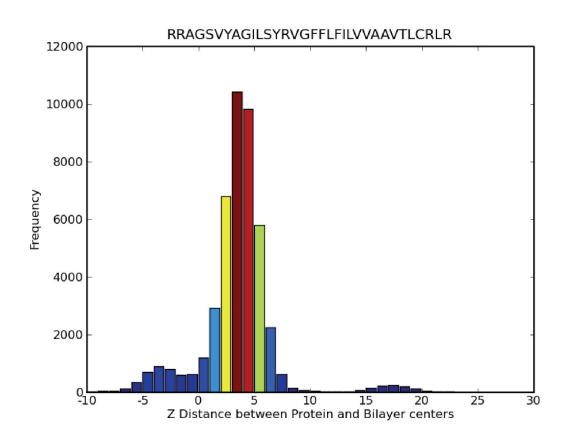


Figure 5.59: The Z separation (along POPC bilayer normal, in Å) between the center of the FGFR3 G380R mutant peptide and the center of the bilayer was recorded across 100 (0.1 μ s) replicate simulations and the frequency distribution is plotted here. The mode of the distribution is an upward (N-terminal) vertical displacement of \sim 4 Å.

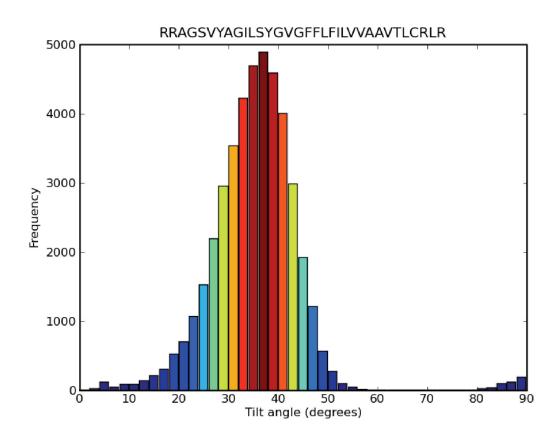


Figure 5.60: The distribution of FGFR3 WT helix tilt angles relative to the bilayer normal is plotted as frequencies accumulated over all the SIDEKICK monomer replicate simulations. The mode of the distribution is a helix tilt angle of $\sim 38^{\circ}$.

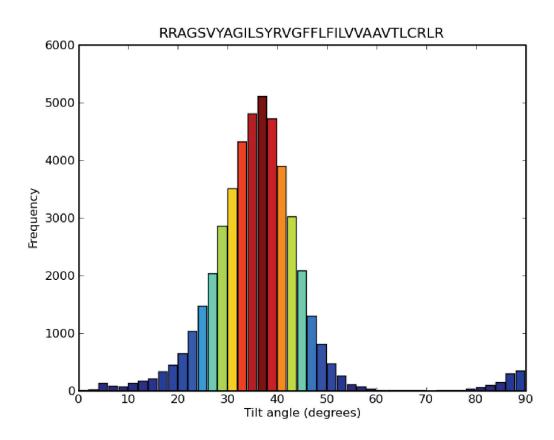


Figure 5.61: The distribution of FGFR3 G380R mutant helix tilt angles relative to the bilayer normal is plotted as frequencies accumulated over all the SIDEKICK monomer replicate simulations. The mode of the distribution is a helix tilt angle of $\sim 38^{\circ}$.

RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR 110 100 90 80

Figure 5.62: The distribution of FGFR3 WT helix rotation angles (about the helical axis) is plotted as frequencies accumulated over all the SIDEKICK monomer replicate simulations.

RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR 110 100 90 80 240 250 260 270 280 290 300

Figure 5.63: The distribution of FGFR3 G380R mutant helix rotation angles (about the helical axis) is plotted as frequencies accumulated over all the SIDEKICK monomer replicate simulations.

Simulation # (cluster used)	${f Description}^a$	$\mathbf{gen_seed}^b$
15 (neuron at SBCB)	WT homodimer replicate #1	117
16 (neuron at SBCB)	WT homodimer replicate #2	7692
17 (neuron at SBCB)	WT homodimer replicate #3	3971
18 (neuron at SBCB)	G380R homodimer replicate #1	38
19 (neuron at SBCB)	G380R homodimer replicate #2	41916
20 (neuron at SBCB)	G380R homodimer replicate #3	5009
21 (neuron at SBCB)	Heterodimer replicate #1	4135
22 (neuron at SBCB)	WT homodimer replicate #4	19109
23 (neuron at SBCB)	WT homodimer replicate #5	6751094
24 (neuron at SBCB)	G380R homodimer replicate #4	43638
25 (mahone at ACEnet)	G380R homodimer replicate #5	13389
26 (mahone at ACEnet)	Heterodimer replicate #2	998120
27 (mahone at ACEnet)	Heterodimer replicate #3	1734
28 (mahone at ACEnet)	Heterodimer replicate #4	581905
29 (mahone at ACEnet)	Heterodimer replicate #5	133786
30 (mahone at ACEnet)	Heterodimer replicate #6	5599878
31 (mahone at ACEnet)	Heterodimer replicate #7	1991
32 (mahone at ACEnet)	Heterodimer replicate #8	2036784
33 (mahone at ACEnet)	Heterodimer replicate #9	556809289
34 (mahone at ACEnet)	Heterodimer replicate #10	13391339
35 (fundy at ACEnet)	G380R homodimer replicate #6	51561673
36 (fundy at ACEnet)	G380R homodimer replicate #7	4666283
37 (fundy at ACEnet)	G380R homodimer replicate #8	4636837
38 (fundy at ACEnet)	G380R homodimer replicate #9	3618616616
39 (fundy at ACEnet)	G380R homodimer replicate #10	447009371
40 (glooscap at ACEnet)	WT homodimer replicate #6	15242205
41 (fundy at ACEnet)	WT homodimer replicate #7	1404083761
42 (glooscap at ACEnet)	WT homodimer replicate #8	255957890
43 (fundy at ACEnet)	WT homodimer replicate #9	89887165
44 (glooscap at ACEnet)	WT homodimer replicate #10	667000009
57 (fundy at ACEnet)	GpA WT control replicate #1	395918
58 (glooscap at ACEnet)	GpA WT control replicate #2	1170019
59 (fundy at ACEnet)	GpA WT control replicate #3	669661
60 (fundy at ACEnet)	GpA WT control replicate #4	454977977
61 (fundy at ACEnet)	GpA WT control replicate #5	393105

Table 5.1: The full set of FGFR3 and GpA dimer replicate simulations as they were tracked during production

 $[^]a$ all simulations involve two (33-residue FGFR3 or 23-residue GpA) peptides in POPC bilayer and water $$^b{\rm GROMACS}$$ starting velocity parameter

Simulation # (cluster used)	${f Description}^a$	$\mathbf{gen}_\mathbf{seed}^b$
47 (fundy at ACEnet)	WT monomer replicate #1	556611119
48 (fundy at ACEnet)	WT monomer replicate #2	23332
49 (fundy at ACEnet)	WT monomer replicate #3	56668990001
50 (fundy at ACEnet)	WT monomer replicate #4	2222913331
51 (fundy at ACEnet)	WT monomer replicate #5	36327
52 (neuron at SBCB)	G380R monomer replicate #1	770091336
53 (neuron at SBCB)	G380R monomer replicate #2	363695
54 (neuron at SBCB)	G380R monomer replicate #3	16443305
55 (neuron at SBCB)	G380R monomer replicate #4	17231355
56 (neuron at SBCB)	G380R monomer replicate #5	6609199

Table 5.2: The full set of FGFR3 monomer replicate simulations as they were tracked during production

 $[^]a$ all simulations involve a single 33-residue FGFR3 peptide in POPC bilayer and water b GROMACS starting velocity parameter

Chapter 6

Spitz-Rhomboid Simulation

6.1 Introduction

The spitz-rhomboid system was described in section 4.1 (page 58) as a prelude to describing production and purification of spitz (or homologous) TMD peptide constructs. The latter project resulted in several successfully synthesized peptides that proved extremely difficult to purify. While it was possible to collect NMR spectra for one construct, it was not possible to fully assign resonances and determine the high-resolution structure. In this chapter I use coarse-grained molecular dynamics simulations to study the interaction of E. coli GlpG (ecGlpG) and a spitz construct in a POPE bilayer. POPE has been used because ecGlpG is active when reconstituted in PE lipids but not in PC lipids (199). The simulation setup and analysis is very similar to that described in chapter 5 (page 108) for FGFR3. Because the methodology and source code are detailed in the previous chapter, I will not be as comprehensive in describing similar methods employed in this chapter. A simple objective of these CG-MD spitz-rhomboid studies is to determine if there is a preferential interaction face between enzyme and substrate (i.e., do they preferentially interact near TM5 the putative substrate gate (148)?) Atomistic simulations have been reported for ecGlpG in POPC and POPE bilayers, but the time scales were short and substrate was not included (195). The CG-MD simulations analyzed in this chapter allow for μ s timescales that include both enzyme and substrate in a lipid bilayer. In total, 20 replicates were performed—two sets of $10\times5~\mu$ s replicates with different spitz starting positions relative to ecGlpG to discourage any association bias.

6.2 Tracking Enzyme-Substrate Separation

A CG representation of the 34-residue spitz TMD construct detailed in section 4.3 (page 61) was placed 70 Å from the geometric center of coarse-grained ecGlpG (PDB: 2IC8, (4)) and the closest C_{α} interprotein separation between the two constructs was monitored. While there were replicates where spitz rapidly associated with TM5 (*i.e.*, Figure 6.1 on page 196), this was not normally observed (*i.e.*, Figure 6.2 on page 197). Thus, the spitz TMD construct does not appear to preferentially associate with TM5 despite evidence that TM5 serves as the substrate gate (148).

6.3 Position Of Spitz In Fixed Rhomboid Reference Frame

The previous section (6.2 on page 192) suggests that spitz does not preferentially associate with TM5 when it first encounters rhomboid, but it is cumbersome to assess the preferred position of spitz over all replicate simulations using the described plots. To gauge the position of spitz relative to each ecGlpG TM segment over all replicate simulations, the configuration of ecGlpG was rmsd-fixed to a reference structure and the positional probability of the geometric center of the spitz TMD construct was monitored in this reference frame. To avoid bias, this analysis was performed for ten replicate simulations where spitz was placed nearer to ecGlpG TMs 1 and 3, or in the opposite corner and nearer TM5 but the same distance from the geometric center of ecGlpG (Figure 6.3 on page 198). Although placing spitz nearer TM5 did increase the likelihood of association at that location, the preferred location of interaction was near ecGlpG TM1 for both starting positions. It may be possible that initial interaction (capture) between enzyme and substrate occurs near TM1 even if the actual gate is

on the other side of the enzyme. In addition, both starting configurations clearly allow for interaction of spitz with various rhomboid TM segments, and this sampling is consistent with an unbiased simulation setup.

6.4 Analysis Of The POPE Lipid Bilayer

The previous section (6.3 on page 192) provides evidence for unbiased simulation conditions and a potential enzyme-substrate interaction site at ecGlpG TM1. However, it is not clear how the lipid bilayer is influenced by the presence of the enzyme, the substrate, and their mutual interaction. As described above, ecGlpG activity is sensitive to the lipid headgroup type when reconstituted, and ecGlpG atomistic simulations suggest a ~4 Å thinning of the bilayer in the vicinity of the enzyme (but not the substrate) (195). Before investigating the protein-local and -distal bilayer thickness, I tested for spontaneous phospholipid flip-flop between bilayer leaflets to ensure that I could use the strategy for bilayer thickness analysis detailed in section 5.5 on page 126 for FGFR3. None of the ten tested replicates (spitz starting near ecGlpG TMs 1 and 3) exhibited flip-flop activity (not shown). I also validated that counting lipids in a local shell within 16 Å of the proteins captured a sufficiently large number of phosphates to measure the leaflet interphosphate bilayer thickness proximal to the proteins (not shown). The average protein-local (within 16 Å) and -distal bilayer thickness values are summarized in Figure 6.4 on page 199. The results are reasonably consistent with the ${\sim}4$ Å proximal bilayer thinning reported for ecGlpG in atomistic bilayers (195), while less bilayer thinning is observed near spitz until it associates with rhomboid. The larger standard deviation observed for spitz-proximal bilayer thickness relative to ecGlpG is consistent with a smaller number of captured (local) lipids in the former versus the latter case because of their vast size difference. There is no additional thinning of the bilayer near ecGlpG after association with spitz, despite the suggestion in (195) that this may occur.

6.5 Identification Of Predominant Interprotein Contacts

There is additional confidence in the quality of the CG simulations after confirming that they are unbiased and that the CG lipids reflect the protein-local behaviour observed in an atomistic context. I began a more in-depth analysis of the interaction between the spitz construct and ecGlpG by parsing out the predominant interprotein contacts from a set of replicate simulations as detailed for FGFR3 in section 5.4.5 (page 116). The results are summarized for each of the spitz construct residues from the first ten replicate simulations (spitz starting near ecGlpG TMs 1 and 3) in Figure 6.5 on page 200. The predominant contacts are located at the N- and C-termini and around the putative ASIASGA consensus sequence (200). It should be noted that the substrate consensus sequence for cleavage is now known to be substantially less specific (141).

Having identified spitz construct residues which interact with ecGlpG, the reciprocal question arises—which residues on ecGlpG interact with the substrate? Using the same methodology for the much larger rhomboid construct produces a cumbersome set of contact probability plots for each of the TM segments and loops (not shown). A more natural approach includes a third dimension to indicate the bilayer burial depth of the residue because this information helps gauge the topological (rather than merely sequential) context within ecGlpG. A representative contact plot for the first ecGlpG helix is shown in Figure 6.6 on page 201, and it is a common theme among most ecGlpG TM segments that the N- and C-terminal residues are predominantly involved in close contacts with the substrate. This is not surprising given that the spitz construct mirrors—with N- and C-terminal residues primarily involved. It was also striking that helix 4 (which contains the catalytic Ser) had almost no contacts with the substrate (not shown), and this is consistent with its protected position in the center of the protease. The full set of ecGlpG top contacts (with the spitz TMD

construct) are summarized in the structure in Figure 6.7 (page 202). It is clear that the predominant interfacial contacts are located at the N- and C-terminal ends of TM segments and in loops between TM segments.

6.6 Conclusions

Despite recent evidence that ecGlpG TM5 serves as the substrate gate (148, 149), the CG-MD simulations indicate that a preferential interaction may occur between spitz and ecGlpG TM1. The spitz TMD construct was able to sample several ecGlpG interaction faces—consistent with an unbiased simulation setup regardless of the precise starting configuration. Protein-lipid interactions were investigated because of the sensitivity of the enzyme to headgroup type, and the ~4 Å bilayer thinning we observe near ecGlpG is consistent with reported atomistic simulations (195), providing confidence in the retention of crucial behaviours despite the simplifications introduced by the CG model. The CG approach provides the unique opportunity to probe the interactions between enzyme and substrate, and my analysis suggests that the predominant interfacial contacts are at the N- and C- terminal ends of ecGlpG TM segments and in loops connecting the TM segments. The latter results were consistent with predominant interfacial residues on the substrate located at the N- and C- termini. The initial capture of substrate by ecGlpG may thus depend on interactions in the juxtamembrane region rather than more central TM residues, and this is consistent with substrate cleavage sequence competency defined near the N-terminal juxtamembrane region (of type I TM proteins) (141). Additional analysis will include the contact probability filtered by polar angle (see section 5.4.9 on page 123), adaptive Poisson-Boltzmann Solver (APBS) electrostatic analysis, and assessment of ecGlpG rmsd/rmsf during the CG simulations.

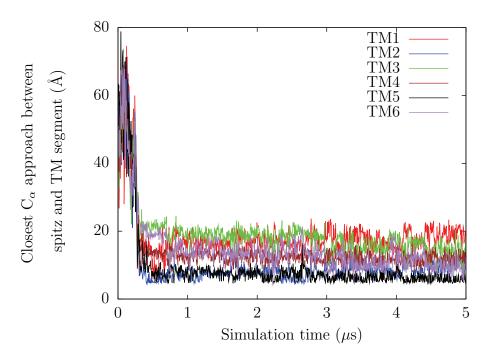


Figure 6.1: The closest interprotein C_{α} separation monitored between spitz and ecGlpG TM segments (as defined in the crystal structure reported in (4)) during the fourth replicate coarse-grained simulation. In this case, spitz associates with TM5 early in the simulation, but this is certainly not observed in most replicates.

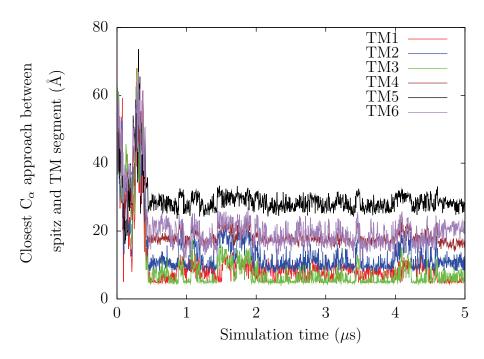
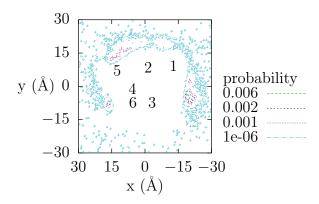


Figure 6.2: The closest interprotein C_{α} separation monitored between spitz and ecGlpG TM segments (as defined in the crystal structure reported in (4)) during the first replicate coarse-grained simulation. In this case, spitz does *not* associate with TM5 early in the simulation, as observed in most replicates.



spitz starting near rhomboid TM 5: $10 \times 5 \mu s$ replicates total

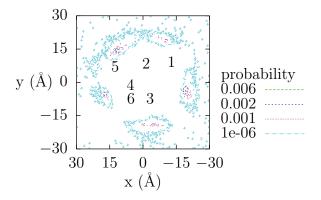


Figure 6.3: The positional probability of the geometric center of the spitz TMD construct is monitored in an rmsd-fixed reference frame (that of rhomboid in the first frame of the first replicate simulation). The ecGlpG TM segment geometric centers are indicated. To avoid any association bias, the starting configuration of spitz was nearer TMs 1 and 3 (top) or TM5 (bottom). Although placing spitz nearer TM5 at the start of the CG simulations appears to increase the likelihood of association at that location, the preferred location of interaction is consistently near TM1. Futhermore, in each case the spitz construct is clearly able to sample multiple interaction faces with rhomboid, and this is consistent with an unbiased interaction between the constructs.

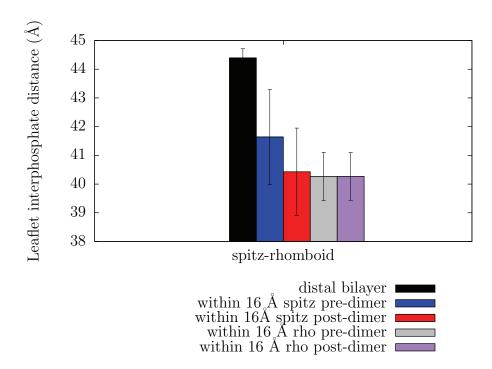


Figure 6.4: Average protein-local and -distal bilayer thickness before and after spitz-rhomboid association (6 Å cutoff) for the ten replicate simulations where spitz starts near ecGlpG TMDs 1 and 3. Error bars display one standard deviation from the mean.

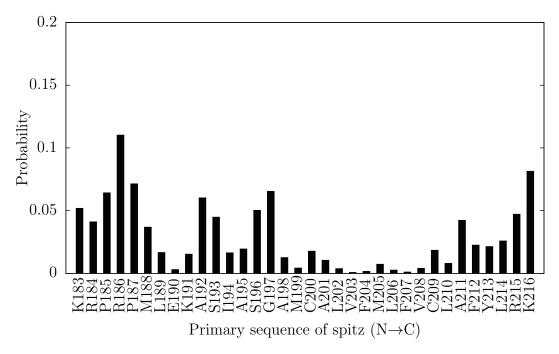


Figure 6.5: The probability for each spitz (TMD construct) residue to reside in the closest contacts with ecGlpG. The exact methodology is described in detail in section 5.4.5 (page 116). The predominant contacts appear to occur at the N- and C-termini, and also around the proposed cleavage consensus sequence (ASIASGA) (200).

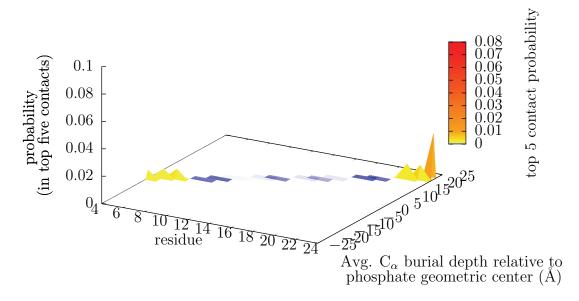


Figure 6.6: The predominant contacts between ecGlpG helix 1 (shown here) and the spitz TMD construct are highlighted and sorted by POPE bilayer burial depth. These results are representative of most ecGlpG helix contact plots, with the N- and C-terminal residues predominantly involved in substrate interaction. The results are aggregated from the first 10 replicate simulations (spitz starting near TMs 1 and 3), and the exact methodology for assessing the predominant contacts is described in detail in section 5.4.5 (page 116)

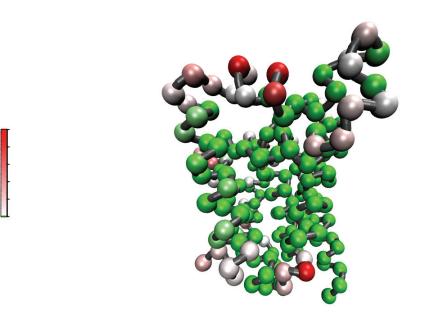


Figure 6.7: The coarse-grained representation of ecGlpG has been simplified to include only C_{α} particles which are coloured based on their close contact probability (see section 5.4.5 on page 116) with the substrate. Water and the lipid bilayer are also excluded for clarity. The probability colour map runs from low (green) \rightarrow medium (white) \rightarrow high (red), with values ranging from 0 \rightarrow 80% (of total close contacts) and is based on the first ten replicate simulations (spitz starting near ecGlpG TM segments 1 and 3). The cytosolic side of ecGlpG is at the bottom and the periplasmic side at the top.

Chapter 7

Conclusions

NMR spectroscopy was used to determine an ensemble of NHE1 TM IX structures in DPC micelles that featured a disruption in helicility near functionally critical residues. A common approach to extend beyond structural information is to probe the flexibility of peptides using NMR spin relaxation experiments. I performed the latter class of experiments for NHE1 TM VII in DPC micelles, and report μ s-ms timescale fluctuations over a critical segment of the peptide. The structural and dynamics results suggest an importance for flexibility in NHE1 TM segments, a theme which has been emphasized in a number of NHE1 TM segment studies (22, 27, 201, 137). It is, however, not yet possible to unambiguously assign the membrane-spanning topology of each NHE1 TM segment to either of the two proposed topologies (13, 14). This will likely require the crystal structure of full-length NHE1.

The poor tractability of spitz or spitz-related rhomboid protease substrate TMD constructs was quite clear. Although many of the constructs were successfully produced by SPPS, purification by HPLC was problematic. When others attempted to produce spitz constructs using expression techniques in *E. coli*, there were also substantial difficulties with yield and purification. In the case of an apparently pure spitz peptide construct, homonuclear NMR spectra were not sufficient to unambiguously assign resonances. Thus, it is worthwhile to include isotope labels in the peptide production process, but there is no clear route to simplified production and purification.

The rhomboid protease system was investigated from another angle using CG-MD simulations, and the initial results suggest that a preferential interaction between enzyme and substrate occurs near TMs 1 and 3 rather than near the proposed substrate gate, TM 5 (148). Furthermore, ecGlpG and the spitz TMD appear to perferentially interact at the terminal ends of helices rather than within the hydrocarbon core of the bilayer.

An extensive analysis of CG-MD simulations of the FGFR3 dimerization process was presented. This includes an integrated discussion of the algorithms used to parse the simulation trajectories, and the appendix includes extensively-documented source code for these analyses. There is no high-resolution structure of FGFR3 available, and these simulation studies provide insight into residues near the dimer interface and the effect of the G380R mutation in the FGFR3 TMD—which causes achondroplasia (202). Strikingly, residue 380 does not feature prominently at the dimer interface, while G370 is one of the closest contacts in the dimers and is mutated to Cys in type 1 thanatophoric dysplasia, a much more severe skeletal phenotype (191). Thus, the phenotypic severity of an FGFR3 mutation may correlate with the proximity of the mutated residue to the dimer interface. I have also described a secondary dimer interface which progressively appears in the heterodimer and mutant homodimer FGFR3 constructs. The rotation of one helix relative to the other may increase signaling activity which causes the phenotype, a conclusion supported by helix-rotation effects reported to affect the activity of receptor tyrosine kinases (197, 198).

Overall, I have employed complementary techniques from structural biology and computational biochemistry to gain insight into biologically relevant membrane proteins.

Appendix A: Source Code For Fractional Isotope Incorporation In Peptide Mass Calculations

A.1 Introduction

rated during solid-phase synthesis. The website employing this code is available at http://129.173.89.133/cgi-bin/isotope -cgi.py. My code has also been incorporated in the back-end of other online mass calculation tools (see http://structbio.bio This appendix contains the Python source code I wrote for peptide mass calculations involving fractional isotope labels incorpochem.dal.ca/jrainey/mspep/).

A.2 Source Code Proper

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```

```
def table_printer(working_list,i,maximum_mass_change,pure_15N_mass,pure_15N_form_input,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Fraction of <br/>br/>synthesis yield</</pre>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 >15</sup>N isotopic enrichment
                                                                                                              +''+'+'+'+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             mass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         0; maintains monoisotropic
                                                        button'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Additional mass from <br/>br/>
                                                                                                                                                                                                                                +''+''+'str(total_mass+(maximum_mass_change-i)+pure_15N_mass(
                                                                                                                                                                      +'</center>'+''+''+''+''+''+'<
                                                       submit
                                                                                                                                                                                                                                                                                                                                                                                                                                      print_isotope_list(fractions_list,total_mass,pure_15N_form_input):
                                                                                                                                                                                                                                                                                         '' +str(total_mass+(maximum_mass_change-i)+pure_15N_mass(
                                                                                                                                                                                                                                                                                                                                                +str(total_mass+(maximum_mass_change-i)+pure_15N_mass(
                                                                                                                                          maximum_mass_change-i+pure_15N_mass(pure_15N_form_input))\
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Total mass
                                                        the
                                                                                                                                                                                                                                                                                                                                                                         pure_15N_form_input)+39)+''''+'</center>'
                                                        hits
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                                                          user
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        working_list.append(1.0-first_fraction) #gain
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             working_list.append(first_fraction) #gains +1
                                                        after the
                                                                                                             print '' +''+str(working_list[i])
                                                                                                                                                                                                   pure_15N_mass(pure_15N_form_input))/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     </
                                                                                                                                                                                                                                                                                                                pure_15N_form_input)+23)+''\
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   '''<center>
                                                                                                                                                                                                                                                          pure_15N_form_input)+1)+''\
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                first_fraction=fractions_list[0]
                                                       the isotope table
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               maximum_mass_change=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     working_list = []
                                                        out
                                                     '''Prints
                              total_mass):
                                                                                                                                                                                                                                                                                                                                                                                                                                    def
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                                                                                                                       101
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```
table_printer(working_list,i,maximum_mass_change,pure_15N_mass
                                                                                                                                                            a single element list stops here
MALDI <br/>br/>H<sup>+</sup>&nbsp;
                                                                                                                                                                                                                                                                                                                                  MALDI <br/> <b
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         new_internal_list.append(new_list[1:-1][x]+new_list
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for x in range(0, len(new_list[1:-1]),2):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              new_list.append(element*fraction)
                                                                                                                                                                                                                                                          Adduct 
                                                                                                                                                                                                                                                                                                                                                                                                                           Adduct 
                                                                                         Adduct 
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              pure_15N_form_input,total_mass)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       i in range(2, len(fractions_list)+1):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           new_list[1:-1]=new_internal_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          else: #more than 1 fractional isotope position
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     in range(0, len(working_list)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for element in working_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 {	t fraction=fractions\_list[i-1]}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       if len(fractions_list)==1: #special case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            working_list=new_list[:]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      [1:-1][x+1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                maximum_mass_change+=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                new_internal_list=[]
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 in range (2):
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```
table_printer(working_list,i,maximum_mass_change,pure_15N_mass,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 form
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       mass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              fraction_input_list=[float(number) for number in
                                              (because
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               decimal numbers separated
                                                                      and last
                                                                                                                                                                                        together the amino acids input by the client and print output'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    15N
                                                                                                                                                                                                                                                                                                                                                        for amino_acid in form.getfirst("client_sequence","").upper():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      non-empty
                                                                                                                                                                 sum_amino_acids(dictionary_used,dictionary_name,pure_15N_form_input):
                                             list elements
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      getfirst("fraction_list").split(',')]
                                                                   same total amu) excluding the first
                                                                                                                                                                                                                                                                                                                                                                                                       total_mass+=18 #include water for the terminal residues
                                                                                                                                                                                                                                        water at some point
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                      isotope incorporation
                                                                                                                                                                                                                                                                                                                                                                                total_mass+=dictionary_used[amino_acid]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    there's
                                            contiguous pairs of
                       pure_15N_form_input,total_mass)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  if form.getfirst("fraction_list"): #if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              a list of
                                                                                                                                                                                                                                                                                                             sequence input
                                                                                                                                                                                                                                       incorporate average mass of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #if it's not
                                                                                                                                                                                                                                                                                                                                                                                                                                                       fractional
                                                                                                                                                                                                                                                                                                            over AA
                                             #add together
                                                                      have the
                                                                                             elements
                                                                                                                                                                                                                                                                                                            iteration
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            fraction list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                except:
                                                                                                                                                                                                                                                                                                                                                                                                                                                       check for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            try:
                                                                                                                                                                                                                                                                                                            try: #try the
                                                                                                                                                                                                                                       have to
                                                                                                                                                                                                                                                                                     total_mass=0
                                                                                                                                                                                                                                       #will
                                                                                                                                                                  def
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```
print '<center><b> Only the 20 common amino acids are accepted as input
                                                                                                                                                           print_isotope_list(fraction_input_list,total_mass,form
 separated
                                                                                               οĮ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   except KeyError: #KeyError could be any text input that is a character other
                                                                                                                                                                                                                                                                                                                                                                                                                   print '<center>'+ dictionary_name + ' : ' + str(total_mass+
                                                                                             list
                                                                                                  ಡ
print '<center><b>Only decimal input values
                                                                                                                                                                                                                                                                                                                                                                                      #no fractional 15N positions; simply add together AAs
                                                                                                                                                                                                                                                                                                                                                                                                                                                  pure_15N_mass(pure_15N_form_input)) + '</center>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          print_form() #call function to print blank or data-entry retained html form
                                                                                          else: #the fractional 15N input was valid; produce
                              by a comma are accepted</b></center>'
                                                                                                                                                                                                                                                                                                                        print '<center>'+ dictionary_name + '</center>'
                                                                                                                                                                                        getfirst('pure_isotopes'))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                than a letter representing one of the common AAs
                                                                                                                              isotope distributions
                                                          sys.exit()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               .</b></center>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          form = cgi.FieldStorage()
                                                                                                                                                                                                                                                                                                                                                                                      else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 189
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```
some
                                                                         sum_amino_acids(monoisotopic_D, "Monoisotopic mass",form.getfirst('
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #so can use getlist to generalize without having to write code to deal with single
                                                                                                                                                             sum_amino_acids(average_D,"Average isotopic mass",form.getfirst('
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              multiple values
                                            if form.getfirst("monoisotopic"): #if the user selects monoisotopic
                                                                                                                                   else: #if the user leaves checkbox empty average masses are used
                 if form.getfirst("client_sequence",""): #if it's not an empty sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             # This way it's safe
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               to enter
                                                                                                                                                                                                                                                                                                                                                                                              code sample for CGI from python std library
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              might try
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        tricky way, or there might be more than one value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               user
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #user = form.getfirst("user", "").upper()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           getfirst because the
                                                                                                      pure_isotopes'))
                                                                                                                                                                                         pure_isotopes'))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #for item in form.getlist("item"):
                                                                                                                                                                                                                    if it's an empty sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #form = cgi.FieldStorage()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                versus multiple values
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     do_something(item)
                                                                                                                                                                                                                                                    total_mass=0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #note the use of
                                                                                                                                                                                                                                                                                                                                                                                                compact
                                                                                                                                                                                                                                                                                                                                                                                                                                                     #import cgi
                                                                                                                                                                                                                         #
                                                                                                                                                                                                                                                                                                                                                                                                  #proper
                                                                                                                                                                                                                          else:
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Appendix B: Additional Data From Spitz Peptide/Protein Production And Purification

- **B.1** Mass Spectrometry Results
- B.1.1 TR-09-1 Construct

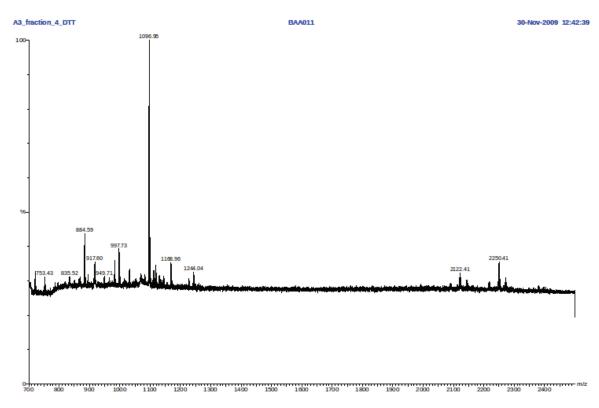


Figure B.1: Fraction 4 from the TR-09-1 crude HPLC run described in Figure 4.12 on page 83 was treated with 200 mM DTT for 1 hour, and then diluted with α -CHC matrix before collecting this reflectron-mode MALDI spectrum. The peak at 2122 m/z is the product missing the N-terminal K while the peak at 2250 m/z corresponds to the desired product. The lower molecular weight compound at 1096 m/z persists in many synthetic spitz fractions.

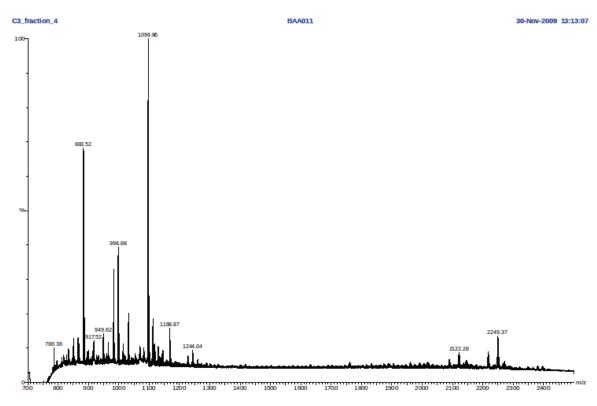


Figure B.2: Similar results are obtained for the TR-09-1 HPLC fraction (#4) used in Figure B.1 (page 217) when there is no DTT pre-treatment.

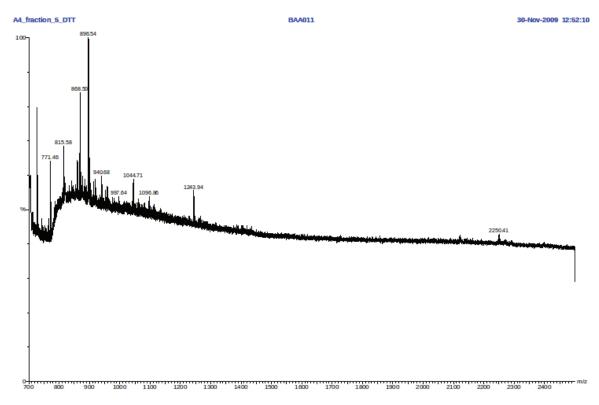


Figure B.3: Fraction 5 from the TR-09-1 crude HPLC run described in Figure 4.12 on page 83 was treated with 200 mM DTT for 1 hour, and then diluted with α -CHC matrix before collecting this reflectron-mode MALDI spectrum. The peak at 2250 m/z corresponds to the desired product, but there are a number of lower molecular weight impurities visible.

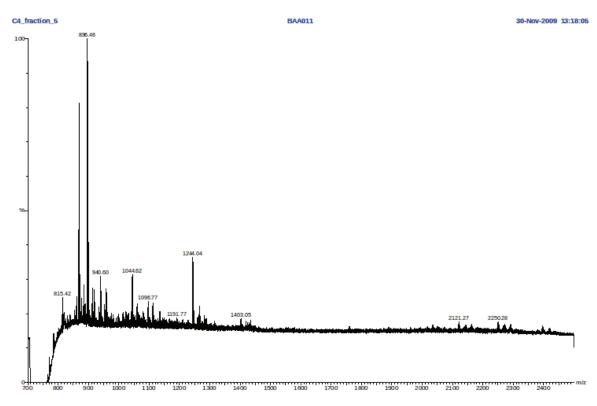


Figure B.4: Similar results are obtained for the TR-09-1 HPLC fraction (#5) used in Figure B.3 (page 219) when there is no DTT pre-treatment.

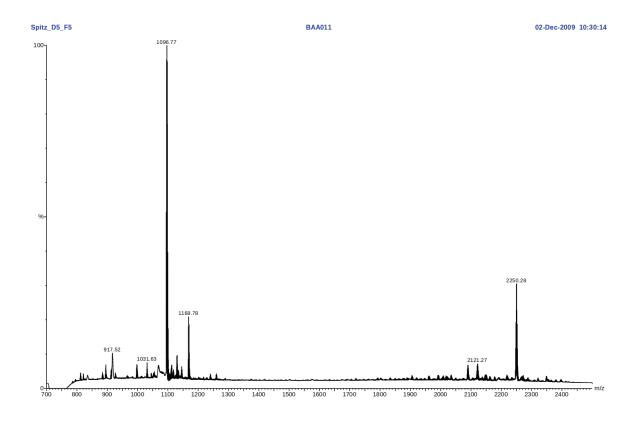


Figure B.5: Reflectron-mode MALDI mass spectrum for fraction 5 (collected between 22-23 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. This is the most promising fraction from the latter HPLC run on the basis of the ratio of the desired product (\sim 2250 m/z) to the product missing the N-terminal K at \sim 2121 m/z (assuming the species have similar ionization potentials).

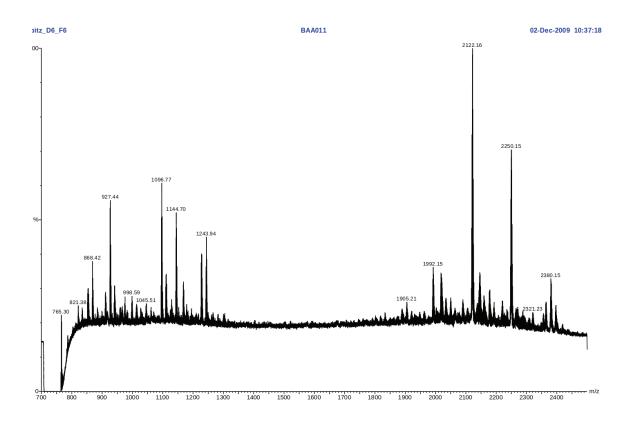


Figure B.6: Reflectron-mode MALDI mass spectrum for fraction 6 (collected between 23-24 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. The desired product is at $\sim\!2250$ m/z while the compound at $\sim\!2122$ m/z is missing the N-terminal K.

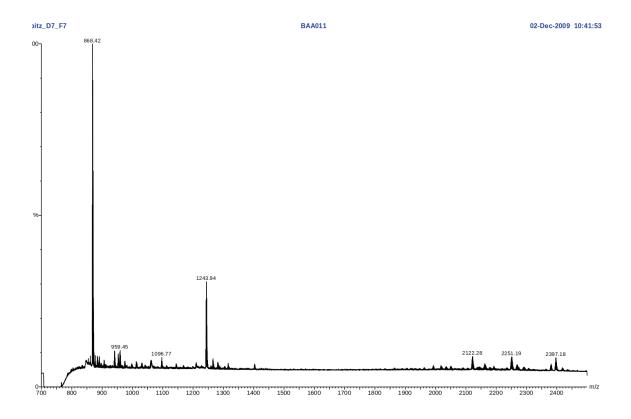


Figure B.7: Reflectron-mode MALDI mass spectrum for fraction 7 (collected between 24-25 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. The desired product is at $\sim\!2250$ m/z while the compound at $\sim\!2122$ m/z is missing the N-terminal K.

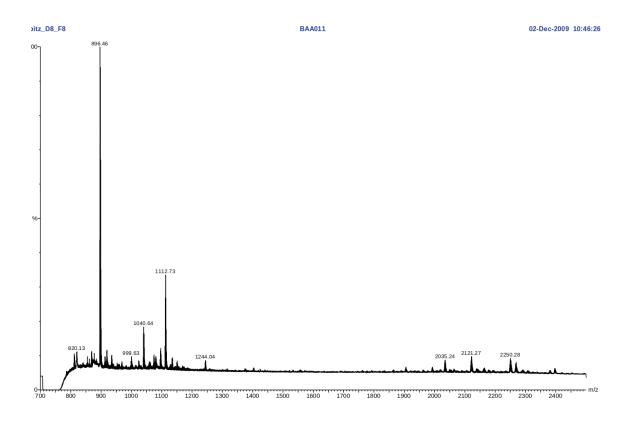


Figure B.8: Reflectron-mode MALDI mass spectrum for fraction 8 (collected between 25-26 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. The desired product is at $\sim\!2250$ m/z while the compound at $\sim\!2122$ m/z is missing the N-terminal K.

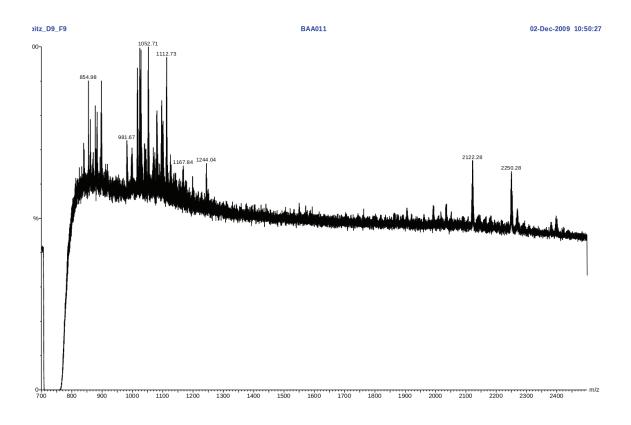


Figure B.9: Reflectron-mode MALDI mass spectrum for fraction 9 (collected between 26-27 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. The desired product is at $\sim\!2250$ m/z while the compound at $\sim\!2122$ m/z is missing the N-terminal K.

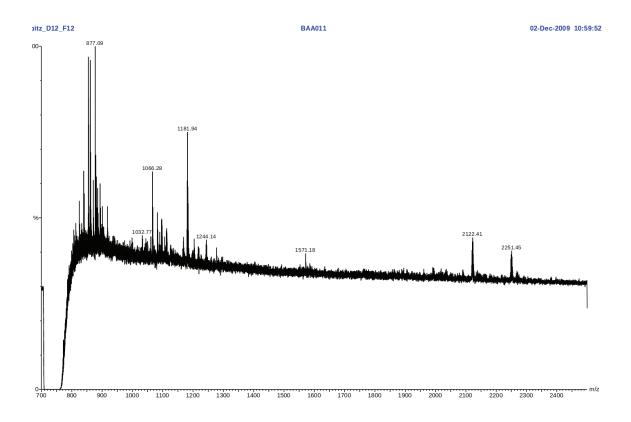


Figure B.10: Reflectron-mode MALDI mass spectrum for fraction 12 (collected between 29-30 minutes) from the crude TR-09-1 HPLC run detailed in Figure 4.13 on page 84. The desired product is at $\sim\!\!2250$ m/z while the compound at $\sim\!\!2122$ m/z is missing the N-terminal K. The product is still eluting 12 minutes after initial detection from the HPLC trace.

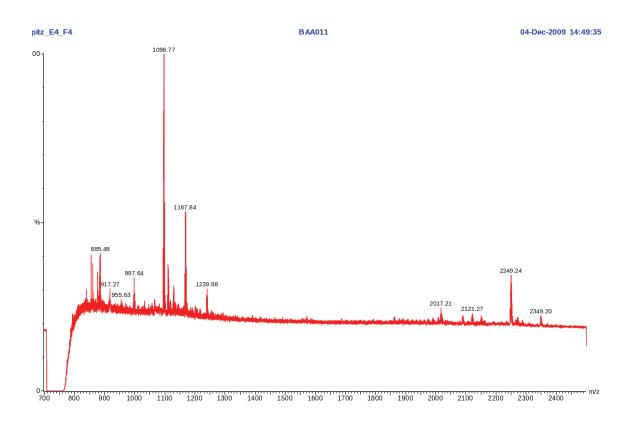


Figure B.11: Fraction #4 (25:01-25:16) from the TR-09-1 C18 HPLC run detailed in Figure 4.15 on page 86 was diluted two-fold with α -CHC matrix and a MALDI spectrum was collected in reflectron mode.

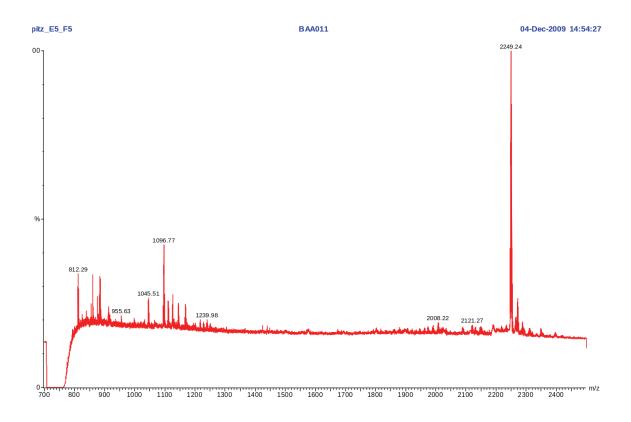


Figure B.12: Fraction #5 (25:16-25:31) from the TR-09-1 C18 HPLC run detailed in Figure 4.15 on page 86 was diluted two-fold with α -CHC matrix and a MALDI spectrum was collected in reflectron mode. This is the highest purity TR-09-1 sample obtained to date, with the product peak at \sim 2249 dwarfing most impurities.

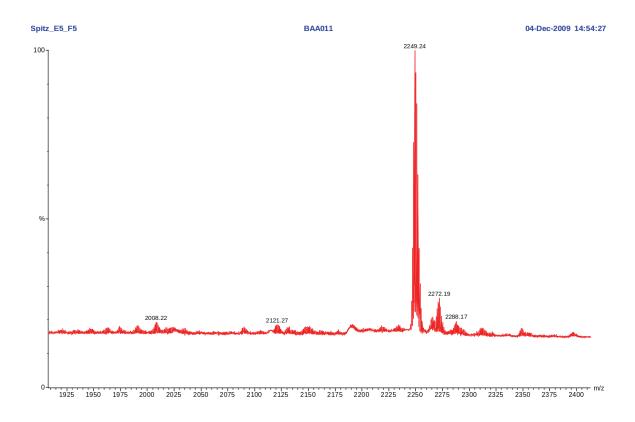


Figure B.13: A zoom-in view of the MALDI spectrum of fraction #5 from Figure B.12 on page 228. The Na $^+$ and K $^+$ adducts of the TR-09-1 product are visible at 2272 m/z and 2288 m/z respectively.

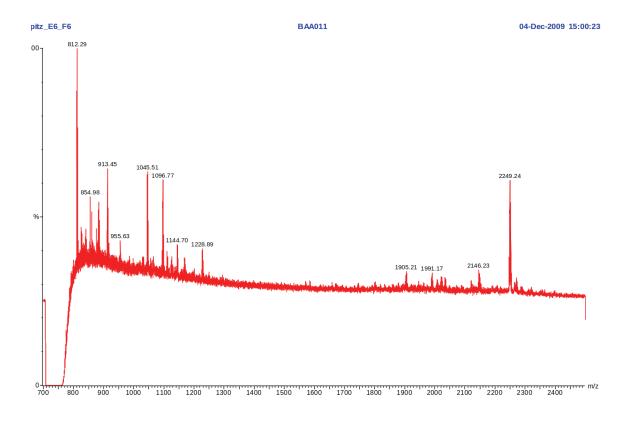


Figure B.14: Fraction #6 (25:31-25:46) from the TR-09-1 C18 HPLC run detailed in Figure 4.15 on page 86 was diluted two-fold with α -CHC matrix and a MALDI spectrum was collected in reflectron mode.

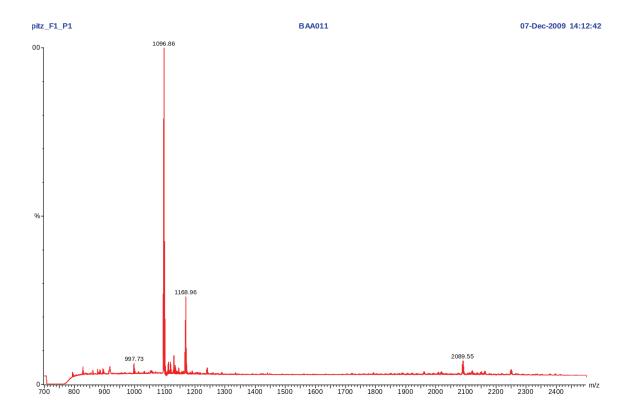


Figure B.15: Fraction #1 from the TR-09-1 C18 semi-preparative HPLC run detailed in Figure 4.17 (page 88) was diluted two-fold with α -CHC matrix and the MALDI spectrum was collected in reflectron mode. The target peak ~2250 m/z is dwarfed by a low molecular weight impurity ~1096 m/z.

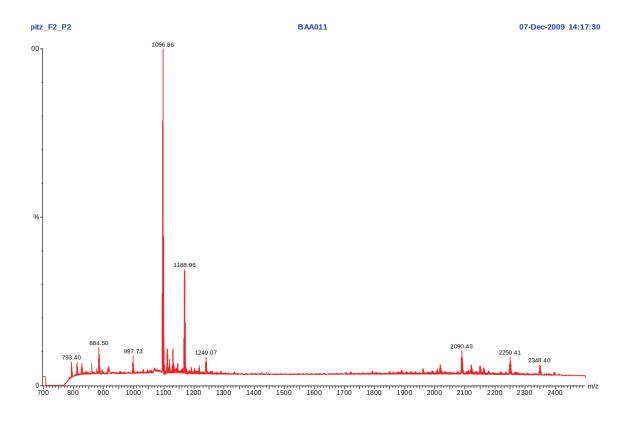


Figure B.16: Fraction #2 from the TR-09-1 C18 semi-preparative HPLC run detailed in Figure 4.17 (page 88) was diluted two-fold with α -CHC matrix and the MALDI spectrum was collected in reflectron mode. The target peak ~2250 m/z is dwarfed by a low molecular weight impurity ~1096 m/z.

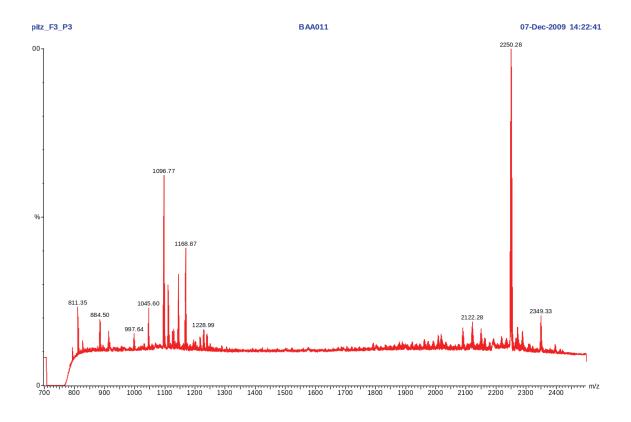


Figure B.17: Fraction #3 from the TR-09-1 C18 semi-preparative HPLC run detailed in Figure 4.17 (page 88) was diluted two-fold with α -CHC matrix and the MALDI spectrum was collected in reflectron mode. The target peak ~2250 m/z is the major peak in the spectrum.

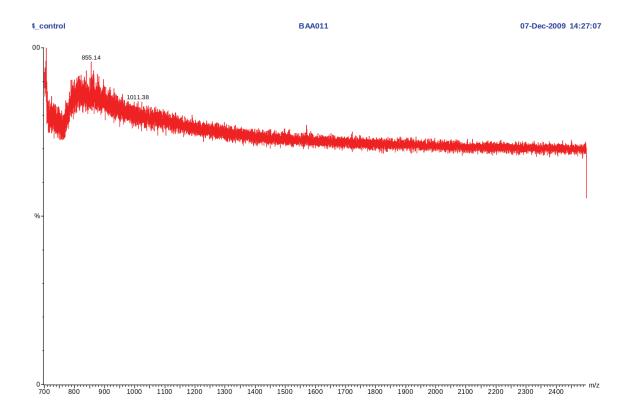


Figure B.18: A blank solution composed of 50% $\rm H_2O/ACN~(0.1\%~TFA)$ was combined with α -CHC matrix to test for low molecular weight impurities which may reside in either the solvents or the matrix. For consistency with test results, the MALDI spectra were collected over the same m/z range in reflectron mode.

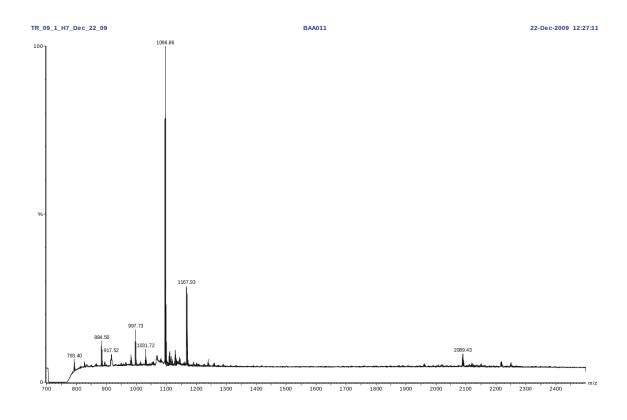


Figure B.19: This is the reflectron-mode MALDI spectrum of a representative fraction (26:40-26:55) from the first crude TR-09-1 bulk purification HPLC run detailed in Figure 4.21 on page 92. The fraction was diluted two-fold with α -CHC matrix prior to collection of the spectrum. The ubiquitous impurity at ~1096 m/z dwarfs the target product at ~2250 m/z.

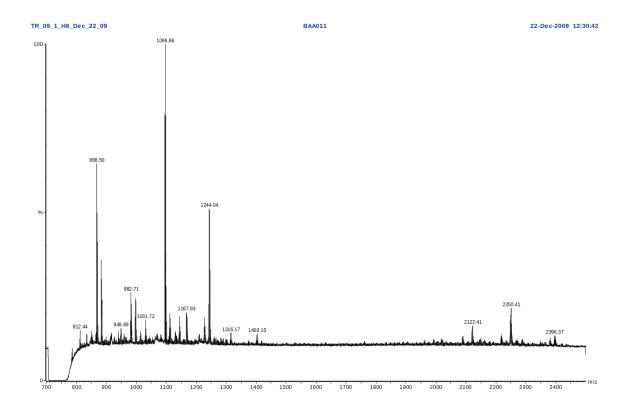


Figure B.20: The most promising MALDI-MS result (shown here) for the first TR-09-1 bulk purification run detailed in Figure 4.21 on page 92 actually correponds to the recovery solution of eluent that flanks the collected fractions between 22-30 minutes. Given the apparent viscosity of the crude TR-09-1 preparation and the trace amount of target product in the collected fractions (see summary Table 4.5 on page 104 and a representative result in Figure B.19 on page 235) it appeares likely that I need to collect fractions later in the HPLC run.

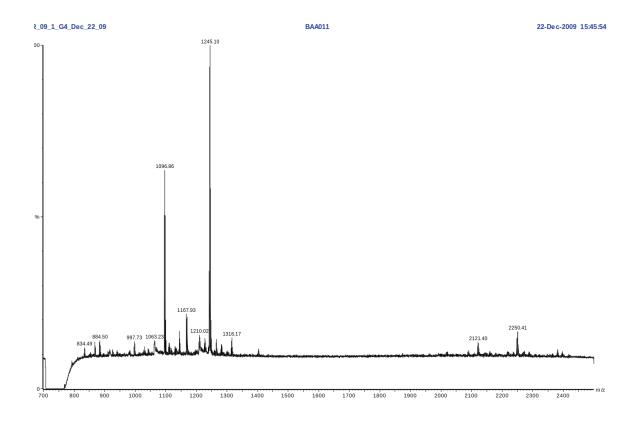


Figure B.21: MALDI reflectron-mode spectrum for the first fraction collected (26:55-27:25) from the second TR-09-1 bulk purification run detailed in Figure 4.21 on page 92. The target product ~2250 m/z appears to be present in greater abundance than the truncated product at ~2121 m/z, but both are dwarfed by lower molecular weight impurities.

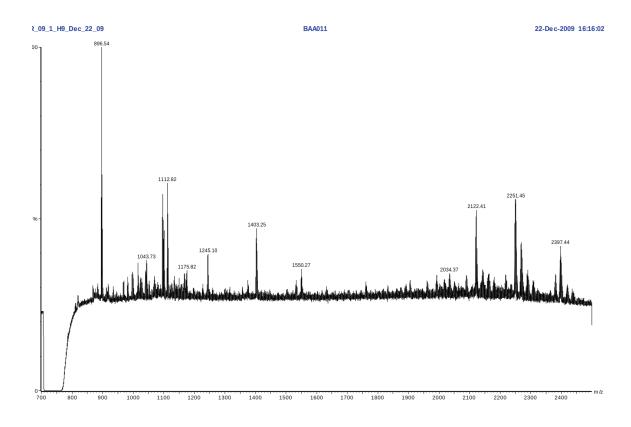


Figure B.22: MALDI reflectron-mode spectrum for the tenth fraction collected (31:25-31:55) from the second TR-09-1 bulk purification run detailed in Figure 4.21 on page 92. This is one of the better results (as summarized in Table 4.6 on page 105) with a fairly prominent ~2250 m/z species. Still, low molecular weight impurities and the truncated product (~2122 m/z) persist.

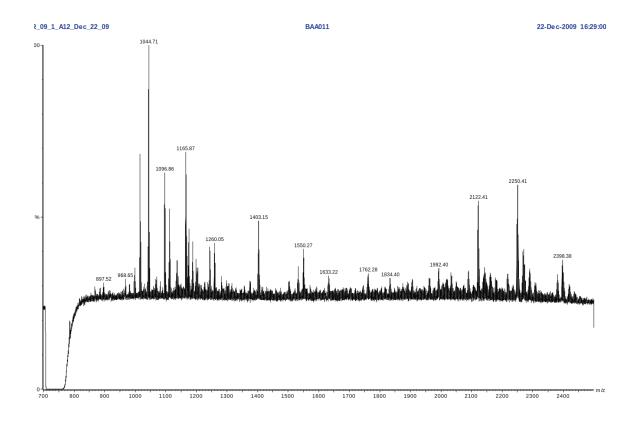


Figure B.23: MALDI reflectron-mode spectrum for the final fraction collected (33:25-33:55) from the second TR-09-1 bulk purification run detailed in Figure 4.21 on page 92. Accounting for the results from the first replicate run (Table 4.5 on page 104), the presence of the target product ~ 2250 m/z here confirms that TR-09-1 elutes from the C18 semi-preparative column over an ~ 8 minute window. This is outrageously poor separation.

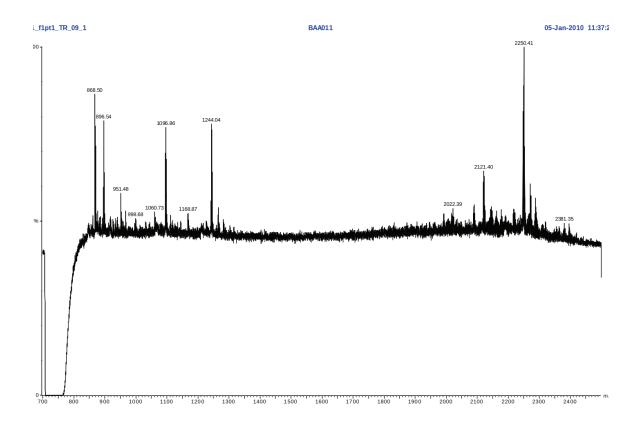


Figure B.24: MALDI reflectron-mode spectrum for the first fraction collected (16-18 minutes) from the TR-09-1 HPLC run detailed in Figure 4.22 on page 93. The target peak $\sim\!\!2250$ m/z is larger than the truncated product at $\sim\!\!2121$ m/z, but (ubiquitous) low molecular weight compounds at $\sim\!\!1096$ m/z and $\sim\!\!1244$ m/z are also co-eluting.

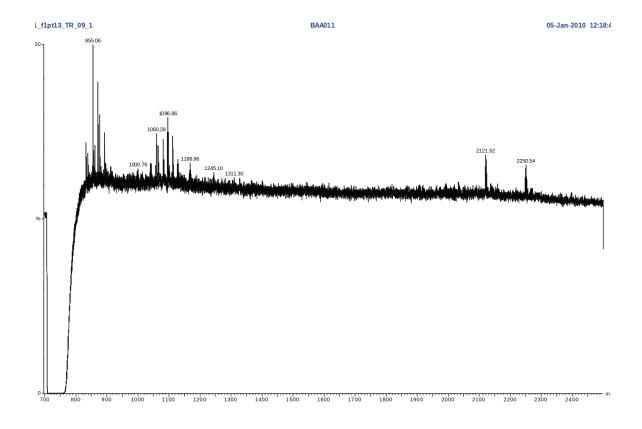


Figure B.25: MALDI reflectron-mode spectrum for the last fraction collected (40-42 minutes) from the TR-09-1 HPLC run detailed in Figure 4.22 on page 93. Despite the fact that this fraction elutes 26 minutes later than the fraction depicted in Figure B.24 (page 240), the product peak at \sim 2250 m/z, the truncated product at \sim 2121 m/z, and low molecular weight impurities at \sim 1096 m/z and \sim 1244 m/z, all persist in the sample.

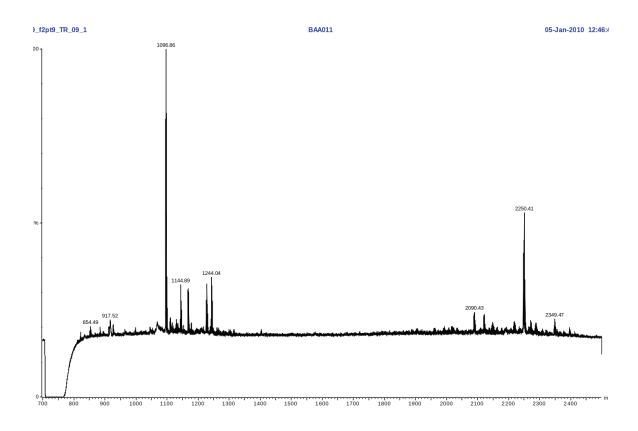


Figure B.26: MALDI reflectron-mode spectrum for the ninth fraction collected (22-24 minutes) from the TR-09-1 HPLC run detailed in Figure 4.23 on page 94. The product peak $\sim\!\!2250$ m/z is prominent, but the ubiquitous low molecular weight compounds at $\sim\!\!1096$ m/z and $\sim\!\!1244$ m/z have clearly co-eluted.

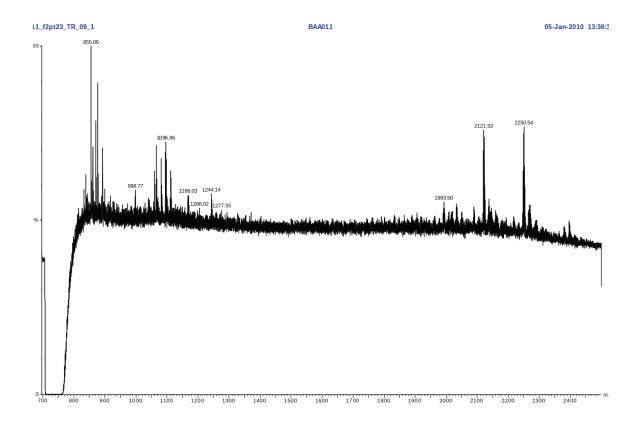


Figure B.27: MALDI reflectron-mode spectrum for the last fraction collected (50-52 minutes) from the TR-09-1 HPLC run detailed in Figure 4.23 on page 94. The product, truncated product, and low molecular weight impurities all continue to elute a full 42 minutes after the inital elution of product (see Table 4.8 on page 106).

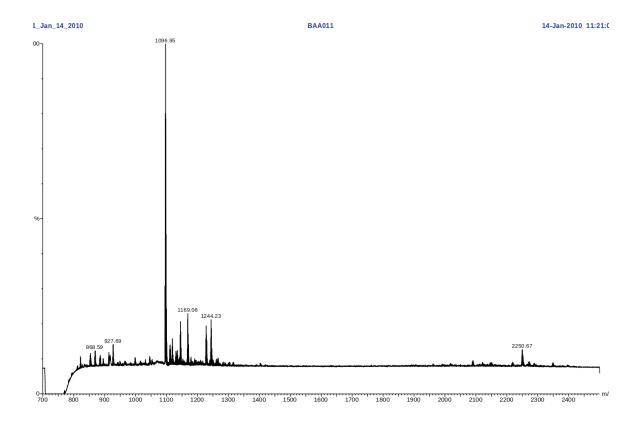


Figure B.28: TR-09-1 fractions collected in the target 22-24 minute window in replicate runs (matching the reference conditions detailed for HPLC purification in Figure 4.23 on page 94) were pooled, lyophilized, and reconstituted in a mixture of deionized water and α -CHC matrix. This reflectron-mode MALDI spectrum is for the first (5.4 mg) of two pooled yields which correctly employed a 2 mL sample loop to match the reference HPLC run. Low molecular weight impurities (most notably the ubiquitous ~1096 m/z species) are present alongside the target product ~2250 m/z.

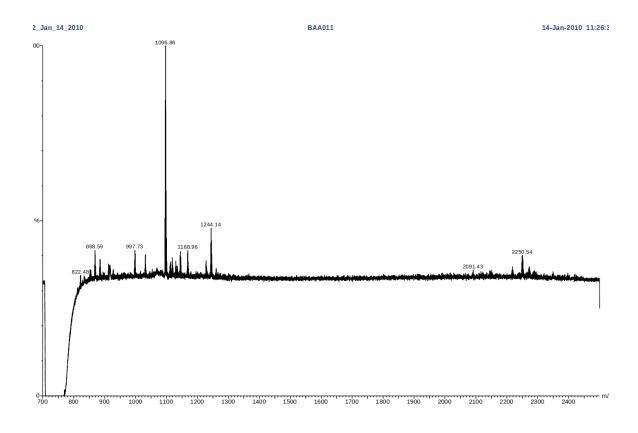


Figure B.29: This is a reflectron-mode MALDI spectrum for pooled TR-09-1 fractions (7.5 mg yield) similar to those described in Figure B.28 (page 244), but collected improperly using a 1 mL sample loop while the reference run employed a 2 mL sample loop. The resulting mass spectrum is effectively the same despite the error, and this is not surprising given the broad elution profile exhibited by TR-09-1.

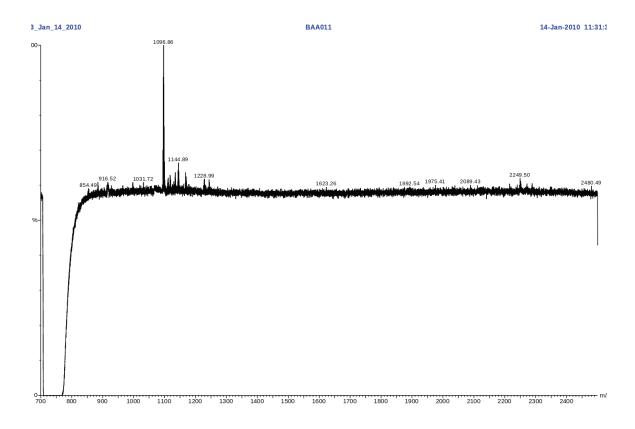


Figure B.30: This reflectron-mode MALDI spectrum was collected for the second pool (11.2 mg yield) of purified TR-09-1 fractions matching the description in Figure B.28 on page 244. Curiously, however, this matching pool of fractions exhibits less target product $\sim\!2250$ m/z relative to the impurity $\sim\!1096$ m/z.

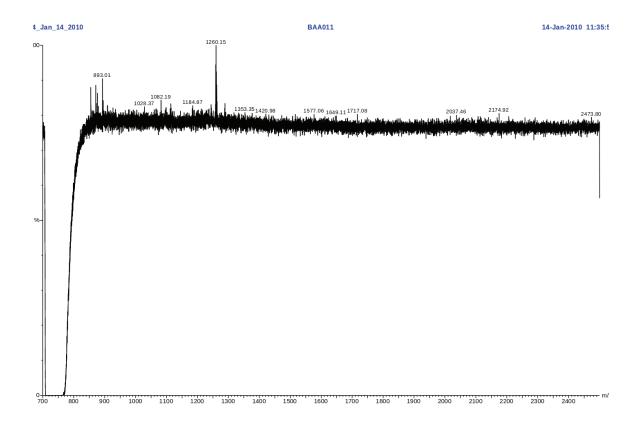


Figure B.31: A 50% DI- $\rm H_2O/\alpha$ -CHC matrix mixture was used as a blank solution for testing the three pooled (partially purified) TR-09-1 fractions above. This MALDI spectrum does not show any substantial indication of the common low molecular weight impurities that permeate the majority of TR-09-1 HPLC fractions.

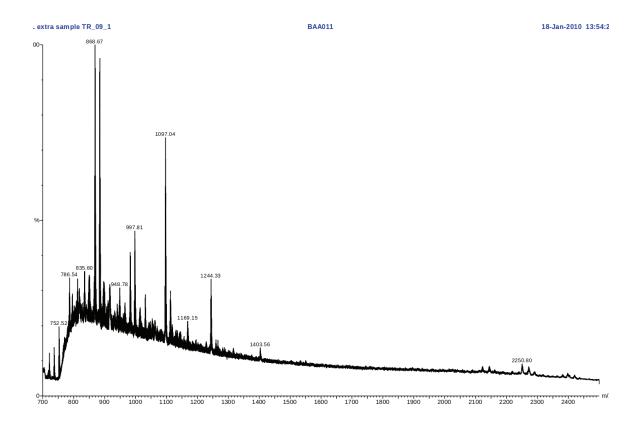


Figure B.32: Eluent collected between 24-52 minutes in TR-09-1 bulk purification runs (which match the reference run detailed in Figure 4.23 on page 94) was lyophilized and reconstituted with deionized water and α -CHC matrix. This reflectron-mode MALDI spectrum demonstrates that the target product ~2250 m/z is still present in the recovered fractions along with the ubiquitous impurities ~1096 m/z and ~1244 m/z.

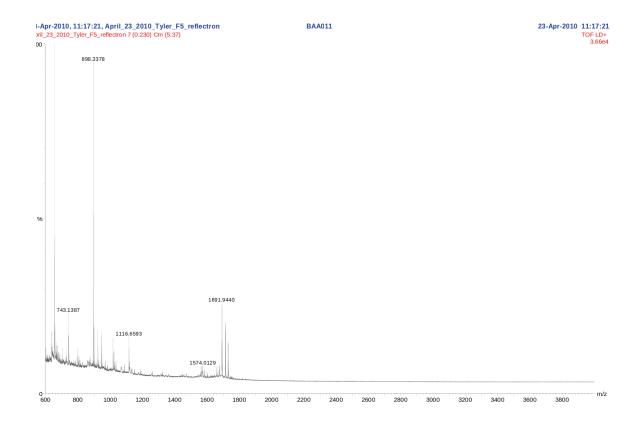


Figure B.33: Fraction 5 (30-36 minutes) from the TR-10-2 HPLC run detailed in Figure 4.27 (page 98) was diluted two-fold with α -CHC matrix and this reflectron-mode MALDI spectrum was collected. The target product is clearly visible ~1691 m/z, and its Na⁺ and K⁺ adducts are also present. However, there is also a low molecular weight impurity ~898 m/z.

B.1.2 TR-10-2 Construct

B.1.3 TR-08-2 Construct

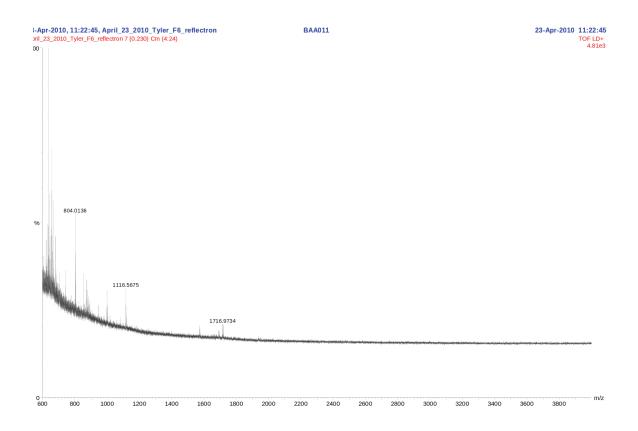


Figure B.34: Fraction 6 (36-42 minutes) from the TR-10-2 HPLC run detailed in Figure 4.27 (page 98) was diluted two-fold with α -CHC matrix and this reflectron-mode MALDI spectrum was collected. The peak \sim 1716 m/z most likely corresponds to the Na⁺ adduct of the target product (\sim 1691 m/z).

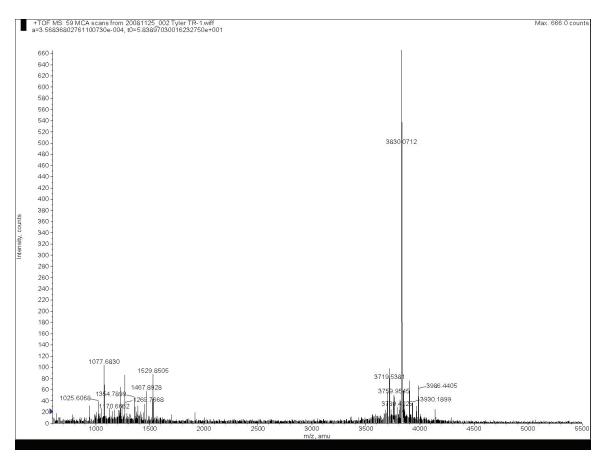


Figure B.35: MALDI spectrum for TR-08-2 fraction #1 from the HPLC run detailed in Figure 4.5 on page 77 demonstrating a relatively pure sample with major peak near the target mass ~ 3830 g/mol.

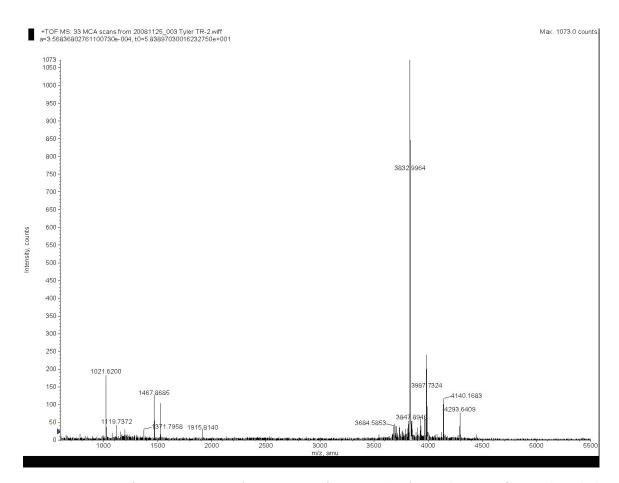


Figure B.36: MALDI spectrum for TR-08-2 fraction #2 from the HPLC run detailed in Figure 4.5 on page 77 demonstrating a relatively pure sample with major peak near the target mass ~ 3830 g/mol. Curiously, a fraction collected earlier in the same HPLC run produced a nearly matching MALDI profile (Figure B.35 on page 251).

Predicts NMR Spin Relaxation Parameter Trends Appendix C: Source Code For Website Which With Magnetic Field Strength

C.1 Introduction

The source code for the online relaxation-plotting program (http://structbio.biochem.dal.ca/jrainey/Tyler_relaxation/) not included all of the front-end html and css code for the website, but the source for the mathematical back-end and some of is written in the Python programming language and uses the cgi module to interact with the web interface user input. I have the user forms are detailed over the following pages.

C.2 Source Code Proper

```
magnetic
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       \texttt{d=math.sqrt((0.1*(gyroH**2)*(gyroN**2)*((6.62606896*(10**-34))**2))/(4*(math.pi**2)*((6.62606896*(10**-34))**2))}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               return (S_squared*tau_m)/(1+((Larmor_value**2)*(tau_m**2)))+((1.0-
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  S_squared)*(1.0/(1.0/tau_m+1.0/tau_e)))/(1+(Larmor_value**2)
                                                                                                                                                                                                                                                         and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    \sharp the constant associated with CSA is a function of external field strength(B)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     def J(tau_m, Larmor_value, S_squared, tau_e, model="classical", S_squared_fast=1,
                                                                                                                                          often used in literature
                                                                                                                                                                                                                                                       gyromagnetic ratio (g)
                                                                                                                                                                                                                                                                                                                                                                                                                                      #define dipolar and CSA constants using Farrow et al., 1994 conventions:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         return math.sqrt((2.0/15)*(gyroN**2)*(B**2)*(CSA**2))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       *((1.0/(1.0/tau_m+1.0/tau_e))**2))
                                                                                                                                      #length of N-H bond vector
                                                                                                                                                                                                                                                         given
                                                                                                     #gyromagnetic ratio of 15N
                                                                                                                                                                             #chemical shift anisotropy
                                                                    #gyromagnetic ratio of 1H
                                                                                                                                                                                                                                                       #function to compute Larmor frequency in s-1
                                                                                                                                                                                                                                                                                                                                                              return (g*B)/(2*math.pi)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           elif model == "extended":
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             if model=="classical":
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #spectral density functions:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            rNH**6)))*(10**-7)
                                                                                                                                                                                                                                                                                          field strength (B)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         def CSA_constant(B):
                                                                gyroH = 2.6752*(10**8)
                                                                                                   gyroN = -2.712*(10**7)
                                                                                                                                          rNH = 1.02*(10**-10)
                                                                                                                                                                             CSA = -160*(10**-6)
                                                                                                                                                                                                                                                                                                                               def Larmor(g,B):
import math
                                                                                                                                                                                                                                                                                                                                                                                                                                                         254
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            19
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             15
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```

```
S_squared,tau_e,model,S_squared_fast,S_squared_slow,tau_slow,tau_fast)+3.0*J(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             S_squared_slow,tau_slow,tau_fast))+((CSA_constant(B)**2)*J(tau_m,Larmor(gyroN
                                                                                                                                **2))*(1.0/(1.0/tau_m+1.0/tau_slow))/(1+(Larmor_value**2)*((1.0/(1.0/
                                                                     Larmor_value**2)*(tau_m**2)))+((S_squared_fast**2)*(1-S_squared_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Larmor(gyroN,B),S_squared,tau_e,model,S_squared_fast,S_squared_slow,tau_slow
                                                                                                                                                                                                     tau_m+1.0/tau_slow))**2))+(1-S_squared_fast**2)*(1.0/(1.0/tau_m+1.0/
                                                                                                                                                                                                                                                                          tau_fast))/(1+(Larmor_value**2)*((1.0/(1.0/tau_m+1.0/tau_fast))**2))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       S_squared_fast, S_squared_slow,tau_slow,tau_fast)+6*J(tau_m,Larmor(gyroH,B)+
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_fast))+((CSA_constant(B)**2)/6.0)*(3*J(tau_m,Larmor(gyroN,B),S_squared,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_e, model, S_squared_fast, S_squared_slow, tau_slow, tau_fast) +4*J(tau_m,0.0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               S_squared_fast,S_squared_slow,tau_slow,tau_fast)+3*J(tau_m,Larmor(gyroN,B),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             S_squared,tau_e,model,S_squared_fast,S_squared_slow,tau_slow,tau_fast)+6*J(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               tau_m ,Larmor(gyroH,B)+Larmor(gyroN,B),S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ,B),S_squared,tau_e,model,S_squared_fast,S_squared_slow,tau_slow,tau_fast))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     S_squared_slow,tau_slow,tau_fast)+J(tau_m,Larmor(gyroH,B)-Larmor(gyroN,B),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          return (d**2)*(J(tau_m, Larmor(gyroH, B)-Larmor(gyroN, B), S_squared, tau_e, model
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_m, Larmor(gyroN,B), S_squared,tau_e,model,S_squared_fast,S_squared_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                def R2(tau_m, B, S_squared, tau_e, model="classical", S_squared_fast=1, S_squared_slow=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ,S_squared_fast=1,S_squared_slow=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    return ((d**2)/2.0)*(4.0*J(tau_m,0.0,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          S_squared,tau_e,model,S_squared_fast,S_squared_slow,tau_slow,tau_fast))
return ((S_squared_fast**2)*(S_squared_slow**2)*(tau_m))/(1+((
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_slow,tau_fast)+6*J(tau_m,Larmor(gyroH,B),S_squared,tau_e,model,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    check with Maple: #print R1(10**-8,11.7,0.85,50*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                    #Spin-lattice relaxation equation from Farrow et al., 1994:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #Spin-spin relaxation equation from Farrow et al., 1994:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   def R1(tau_m,B,S_squared,tau_e,model="classical"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        au_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #quality
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   31
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                59
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```
tau_m ,Larmor(gyroH,B)-Larmor(gyroN,B),S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                  def NOE(tau_m, B, S_squared, tau_e, model="classical", S_squared_fast=1, S_squared_slow=1,
                                                                                                                                                                                                                                                                                                                          S_squared, tau_e, model, S_squared_fast, S_squared_slow, tau_slow, tau_fast)-J(
                                                                                                                                                                                                                                                                           return 1+(gyroH/gyroN)*(d**2)*(6*J(tau_m,Larmor(gyroH,B)+Larmor(gyroN,B),
                                                                                                                                                                                                                                                                                                                                                                                                                                           S_squared_slow,tau_slow,tau_fast))*(1/R1(tau_m,B,S_squared,tau_e,model
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       check with Maple: #print NOE(10**-8,11.7,0.85,50*(10**-12))
with Maple: #print R2(10**-8,11.7,0.35,50*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               S_squared_fast,S_squared_slow,tau_slow,tau_fast))
                                                                                                              #Steady-state NOE equation from Farrow et al., 1994:
                                                                                                                                                                                                                       tau_slow=1*(10**-9),tau_fast=1*(10**-12))
     #quality check
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #quality
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    39
                                                                                                                                                                                37
                                                                       35
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```
=10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical", S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                          __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #self.varied_parameter=varied_parameter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #self.varied_parameter=varied_parameter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.npar=npar+1 #number of gradations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #self.relax_parameter=relax_parameter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.parameter_min=parameter_min
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.parameter_max=parameter_max
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #self.unique_name=unique_name
                                                                                                                                                                                                                                                                                                                             #os.putenv('GNUTERM','postscript')
                                                                         from constants_equations import *
                                              import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                       cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                              decimal.getcontext().prec=2
                                                                                                                                                                                                                                                                                                     #os.putenv('DISPLAY',':0')
                                                                                                                                                                                                                                                                                                                                                                                                                                   multi_plotter:
                                                                                                                           decimal
                                                                                                                                                  random
                       math
                                                                                                                                                                                                    time
Sys
                                                                                                                                                                                                                             import csv
                                                                                                  import os
                                                                                                                                                    import
import
                       import
                                                                                                                                                                             import
                                                                                                                                                                                                    import
                                                                                                                           import
                                                                                                                                                                                                                                                                                                                                                                                                                                 class
                                                                                                       n
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                                                                                                                                                                                                                                                           11
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     23
                                                                                                                                                                                                                                                                                                                 257
```

```
self.file_label_list=['<em>T<sub>1</sub></em>','<em>T<sub>2</sub></em>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #abstract superclass -- subclasses fill in the work for the intervening
                                                                                                                                                                                                                                                                                                                                                                                                   ,'<em>NOE</em>','<em>R<sub>1</em>','<em>R<sub>2</em>']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.parameter_value_list.append(self.parameter_min+i*((self
                                                                                                                                                                              self.relax_plot_list=['T_1','T_2','steady-state NOE','R_1','R_2']
self.y_label_list=['T_1 (s)','T_2 (s)','Steady-state NOE','R_1 (s^
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              param values
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  parameter_max-self.parameter_min)/self.npar))
                                                                                                                                                                                                                                                                                       self.file_namer=['T1','T2','NOE','R1','R2']
self.plot_units_list=['(s)','(s)','','(s^-1)','(s^-1)']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.parameter_value_list.append(self.parameter_max)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #generate list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       S_squared_fast=S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        S_squared_slow=S_squared_slow
self.field_values=field_values
                               self.parameter_value_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              in range(self.npar):
                                                                                                                                                                                                                                                                                                                                                                                                                                      #self.S_squared=S_squared
                                                                                                                                           self.plot_object_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      .tau_slow=tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #self.tau_fast=tau_fast
                                                                                                                                                                                                                                                    ','R_2 (s^{-1})']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #self.tau_e=tau_e
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #self.tau_m=tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #self.model=model
                                                                                                       self.plot_list=[]
                                                                  self.L_plot=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ٠,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for
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```
#Generate the physical gnuplot objects that can be plotted by gnuplot:
                                        preparation for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.file_name='%stmp_%d.ps' % (self.name_prefix,os.getpid())
self.input_to_gp='set output "../relaxation_tmp/%s"' % self.file_name
                                                                                                                                                    self.plot_list.append(self.L_plot[0+i*self.field_values:self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.plot_object=Gnuplot.PlotItems.Data(self.plot_list[i],
                                                                                                                                                                                                                                                                                                                                                                                                                     parameter_value_list[i]))*decimal.Decimal('1.00'))+self
                                                                                                                                                                                                                                                                                                                                              #print decimal.Decimal(str(self.parameter_value_list[i]))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   .f.graph_title='set title "Influence of %s on trend of %s with magnetic field strength\\n\\n(%s %s)"' % self.g
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.gp('set key outside bottom box') #position of legend
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  {	t self.gp('set xlabel "Magnetic field strength (Tesla)"')}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.plot_object_list.append(self.plot_object)
                                        parameter in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #control appearance of the plot generated by gnuplot:
                                                                                                                                                                                                                                                                                                                                                                                   title_iteration=str(decimal.Decimal(str(self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       with_='lines', title=title_iteration)
                                                                                                                                                                                                                                                                                                          in range(len(self.parameter_value_list)):
                                                                                                               for i in range(len(self.parameter_value_list))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.gp('set term postscript enhanced color')
                                                                                                                                                                                        field_values+i*self.field_values])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.graph_title='set title "Influence of
                                        #Splice the list in blocks by the varied
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.gp=Gnuplot.Gnuplot(persist=0)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.gp('set data style lines')
                                                                                                                                                                                                                                                                                                                                                                                                                                                              parameter_unit_label
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.gp('set xrange [0:25]')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.gp(self.graph_title)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.gp(self.y_label)
                                                                            plotting:
plot_2(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   22
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self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                          a python list of PlotItems
                                                                                                                                                                                                         code..
                                                                                                                            commas
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.plot_object_list[12],self.plot_object_list[13],self.plot_object_list[14],self.
                                                                                                                                                                                                                                                                                                                         self.plot_object_list[2],self.plot_object_list[3],self.
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                                                                                                                                                                                                                                                                                                                                                                                                            self.plot_object_list[6],self.plot_object_list[7],self.plot_object_list[8],self.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.plot_object_list[6],self.plot_object_list[7],self.plot_object_list[8],self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.plot_object_list[12],self.plot_object_list[13],self.plot_object_list[14])
                                                                                                                      explicit indexed values separated
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.plot_object_list[6],self.plot_object_list[7],self.plot_object_list[8]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           plot_object_list[9], self.plot_object_list[10], self.plot_object_list[11]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   plot_object_list[9],self.plot_object_list[10],self.plot_object_list[11]
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                                                                                                                                                                                                                                                                                                                                                                  plot_object_list[4],self.plot_object_list[5]
                                                                                                                                                                                                       condense this
                                                                            seem to tolerate
                                                                                                                                                                                                       able to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.plot_object_list[12],self.plot_object_list[13])
                                                                                                                                                                                                     to be
                                                                              #curiously gnuplot doesn't
                                                                                                                      Data, but will tolerate
                                                                                                                                                                                                       #would definitely be nice
self.gp(self.input_to_gp)
                                                                                                                                                               as below ... very odd
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              elif self.npar==14:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      elif self.npar==13:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            elif self.npar==12:
                                                                                                                                                                                                                                              if self.npar == 15:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    plot_object_list[15])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        87
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self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                 self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                    self.plot_object_list[2],self.plot_object_list[3],self.
                                             self.plot_object_list[2],self.plot_object_list[3],self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.plot_object_list[2],self.plot_object_list[3],self
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                                                                                                                                               self.plot_object_list[6],self.plot_object_list[7],self.plot_object_list[8],self.
                                                                                                                                                                                               plot_object_list[9], self.plot_object_list[10], self.plot_object_list[11], self.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.plot_object_list[6],self.plot_object_list[7],self.plot_object_list[8]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              plot_object_list[9],self.plot_object_list[10],self.plot_object_list[11])
                                                                                                                                                                                                                                                                                                                                                                                                                                                 plot_object_list[4],self.plot_object_list[5],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       plot_object_list[4],self.plot_object_list[5],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         plot_object_list[4],self.plot_object_list[5],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   plot_object_list[4], self.plot_object_list[5]
                                                                                          plot_object_list[4],self.plot_object_list[5]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     plot_object_list[9],self.plot_object_list[10])
                                                                                                                                                                                                                                                                                                    elif self.npar==11:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      elif self.npar==10:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.npar==8:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              elif self.npar==7:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        elif self.npar==9:
                                                                                                                                                                                                                                               plot_object_list[12])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          plot_object_list[9])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           107
                                                                                                                                                              92
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self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                          self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                 self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.gp.plot(self.plot_object_list[0],self.plot_object_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.gp.plot(self.plot_object_list[0],self.plot_object_list[1])
                                                                                                                                                                                                                             self.plot_object_list[2],self.plot_object_list[3],self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.plot_object_list[2],self.plot_object_list[3],self.
                                      self.plot_object_list[2],self.plot_object_list[3],self
                                                                                                                                                                                                                                                                                                                                                                                                                      self.plot_object_list[2],self.plot_object_list[3],self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  gradations must be between 0 and 14
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.plot_object_list[2],self.plot_object_list[3])
                                                                                                                                                                                                                                                                 plot_object_list[4],self.plot_object_list[5],self
                                                                                                                                                                                                                                                                                                                                                                                                                                                          plot_object_list[4],self.plot_object_list[5])
                                                                       plot_object_list[4],self.plot_object_list[5],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.input_file='../relaxation_tmp/'+self.file_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.plot_object_list[2])
                                                                                                             self.plot_object_list[6],self.plot_object_list[7])
                                                                                                                                                                                                                                                                                                        plot_object_list[6])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                plot_object_list[4])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    print 'Number of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.npar==2:
                                                                                                                                                                                                                                                                                                                                              self.npar == 5:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.npar==4:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.npar==3:
                                                                                                                                                        elif self.npar==6:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.npar == 1:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 else:
         109
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            115
                                                                                                                                                                                                                                                                                                                                                        113
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                119
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               127
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  117
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    125
                                                                                                                                                               111
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                121
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         123
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           262
```

```
time.sleep(1) # the sleep delay (1 second) is needed because on the Red
                                                                                                                                        server it seems that .png files sometimes aren't being produced
self.output_file='../relaxation_tmp/'+self.file_name.replace('ps','png
                                                     (self.input_file,
                                                                                                                                                                                                                      seems to be too short, and missing image
                                                                                                                                                                  from the .ps-->speculating this is because gnuplot isn't closing
                                                                                                                                                                                                                                                                                                                                     self
                                                                                                                                                                                           enough before ImageMagick tries to find the .ps file.
                                                                                                                                                                                                                                                                                                                                  print '<center><img src="/relaxation_tmp%s"></center>' %
                                                   % , s%
                                                   s
%
                                                                                                                                                                                                                                                                                                                                                             output_file.replace('../relaxation_tmp','')
                                                     -rotate 90
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.plot_list_labelled=self.plot_list
                                                   convert_input='/usr/bin/convert
                                                                                                                                                                                                                        #also, shorter than 1 second
                                                                                                                                                                                                                                                  links show up occasionally
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.plot_list_labelled=[]
                                                                                                                                                                                                                                                                             os.system(convert_input)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.plot_object_list=[]
                                                                                  self.output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.position+=520
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.plot_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.set_label=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.L_plot=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.csv_out()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 print '<hr />
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #reset lists
                                                                                                                                                                                                                                                                                                                                                                                                                                              #CSV output:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      csv_out(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          def
                                                                                                                                                                                                                                                                                                                                                                                            263
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            149
                                                             129
                                                                                                                                                                                                                                                                                                              133
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               145
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     147
                                                                                                                                                                                                                               131
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             139
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   141
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        143
```

```
parameter_value_list[i])+''+self.parameter_unit_label+',')
                                                                                                                                              first_line='Field strength (Tesla), %s %s\n' % (self.relax_parameter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #first_line_list=str(self.parameter_value_list[value])+self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.plot_list_labelled[i].append(self.set_label[i+1])
                                                                                                                                                                                                                                                                                             #self.set_label.append(self.varied_parameter+'='+str(self
                                  varied_parameter, os.getpid()+random.randint(0,10**12))
file_string='../relaxation_tmp/%stext_out%d.csv' % (self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   f.write(self.varied_parameter+'='+','+str(self
                                                                                                                                                                                                                                                                                                                                                                                                   string_sub=self.varied_parameter+'='+str(self
                                                                                                                                                                                                                                                                                                                               parameter_value_list[0])+self.parameter_unit_label
                                                                                                                                                                                                                                                                                                                                                                                                                                       parameter_value_list[i])+self.parameter_unit_label
                                                                                                                                                                                                                                                                                                                                                                  #for i in range(1,len(self.parameter_value_list)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #self.plot_list_labelled[0][0:0]=self.set_label[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for i in range(len(self.parameter_value_list)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #f.write(str(self.plot_list_labelled)+'/n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.set_label.append(string_sub)
                                                                                                           csv_f=csv.writer(f,dialect='excel')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #for value in range(self.npar):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            parameter_unit_label
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 i in range(self.npar):
                                                                         f=open(file_string,'w')
                                                                                                                                                                                                                       f.write(first_line)
                                                                                                                                                                                                                                                          #self.set_label=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               f.write('\n')
                                                                                                                                                                                    self.plot_units)
                                                                                                               #
                                                                                                                                                #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       159
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  264^{\frac{79}{91}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          173
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         169
                                                                                  151
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               167
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              171
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```
field_values=26,tau_m=10**-8,S_squared=0.85,tau_e=50*(10**-12),model=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical", S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          data</
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     h4><a href="%s">%s: CSV plot data file</a></div>''' % (self.position
f.write((('Field strength (Tesla), %s %s,' % (self.relax_parameter, self
                                                                                                                                     and
                                                                                                                                                                                                                                       curve for
                                                                                                                                                                                                                                                                                                  row.append(curve[field_value]) #the row variable thus
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           "classical", S_squared_fast=1, S_squared_slow=1, tau_slow=1*(10**-9),
                                                                                                                                                                                                                                                                                                                                      a given x (field
                                                                                                                                     0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        print '''<div class="extra_data" style="top:%dpx"><h4>Additional
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                     M/q
                                                                                                                                                                                                                                                                                                                                                                                                   f.write(str(row).replace('[','').replace(']','')+'\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              multi_plotter.__init__(self,parameter_min,parameter_max,npar,
                                                                                                                                     varies
                                                                                                                                                                                                                                           one
                                                                                                                                                                                                                                     J.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     file_string.replace('../','.','),self.file_label)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                   always
                                                                                                                                                                                                                                     #three
                                                                                                                                                                                                                                                                                                                                        contains the x,y coordinates for
                                                                                                                                                                                                                                     for curve in self.plot_list_labelled:
                                                                                                                                   strength
                                                                                                                                                                                                  row=[] #reset row list each iteration
                                                                                                                                                                                                                                                                                                                                                                   strength) for all curves
                                                                                                                                                                                                                                                                       each set of parameter values
                                                                                                                                range(26): #field
                              plot_units))*(self.npar+1))+' \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.parameter_unit_label='
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ш
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          tau_m_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.symbol='{/Symbol t}_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.value=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.i=5 #arbitrary
                                                                                                                                   for field_value in
                                                                                                                                                                         25 Tesla
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    185
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           \frac{3}{2}65
                                                                          175
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           191
                                                                                                                                            177
                                                                                                                                                                                                                                              179
                                                                                                                                                                                                                                                                                                                                                                                                               181
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     187
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         189
```

```
tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  S_squared_slow,tau_slow,tau_fast),1.0/R2(self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   .tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ,S_squared_slow,tau_slow,tau_fast),NOE(self
                                                                                                                                                                                              plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           S_squared_slow, tau_slow, tau_fast), R2(self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              S_squared_slow, tau_slow, tau_fast), R1(self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.L_plot.append([i,[1.0/R1(self.tau_m,i,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      S_squared_slow,tau_slow,tau_fast)][j]])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              S_squared, tau_e, model, S_squared_fast,
                                                                                                                                                                                                                                             S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                      S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)): #plot
                                                                                                                                                                                                                                                                                                                                       handler so call it to run plot 1 and plot 2 for a given instance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        for self.tau_m in self.parameter_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.relax_parameter=self.relax_plot_list[j]
                                                {/Symbol t}_e'+'='+str(tau_e)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 for i in range(self.field_values):
self.constant_1='S^2'+'='+str(S_squared)
                                                                                               self.varied_parameter='tau_m'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.constant_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      (0,10**12))
                                                                                                                                                                                                                                                                                                                                                                                                                                         for j in range(5):
                                              self.constant_2=
                                                                                                                                                                                                                                                                                                                                                                                           self.position=10
```

193

195

197

199

266⁸

```
field_values=26,tau_m=10**-8,S_squared=0.85,tau_e=50*(10**-12),model=
                                                                                                                                                                                                                                           ,S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   def plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                      "classical", S_squared_fast=1, S_squared_slow=1, tau_slow=1*(10**-9),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)): #plot 1 is
                                                                                                                                                                                                      __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                        multi_plotter.__init__(self,parameter_min,parameter_max,npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       handler so call it to run plot 1 and plot 2 for a given instance
                                                                                                                                                                                                                                      =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical"
                                                                                                                                                                                                                                                                                 S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for self.tau_e in self.parameter_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.constant_2=\ \{/Symbol t\}_m'+'='+str(tau_m)+'
self.plot_units=self.plot_units_list[j]
                                  self.file_label=self.file_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.constant_1='S^2'+'='+str(S_squared)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.parameter_unit_label=' s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.varied_parameter='tau_e'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.symbol='{/Symbol t}_e'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.constant_2)
                                                                                                                                                             tau_e_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                             tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.value=5 #arbitrary
                                                                           self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       for j in range(5):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.position=10
                                                                                                                                                                 class
                                                                                                                                                                     211
        207
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           215
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                                                                                       209
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        219
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      221
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```
field_values=26, tau_m=10**-8, S_squared=0.85, tau_e=50*(10**-12), model= \frac{1}{2}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         , S_squared_fast=1,
                                      self.L_plot.append([i,[1.0/R1(tau_m,i,S_squared
                                                                                                                                                                                                                                                                                           model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                              model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                                                                                                                                             model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                 tau_fast), NOE(tau_m,i,S_squared,self.tau_e,
                                                                                                                                                                                                                                                                                                                                                                                                                      tau_fast), R2(tau_m,i,S_squared,self.tau_e,
                                                                                                                                                                                                                                                                                                                                 tau_fast), R1(tau_m,i,S_squared,self.tau_e,
                                                                                                                    S_squared_slow,tau_slow,tau_fast),1.0/R2(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           "classical",S_squared_fast=1,S_squared_slow=1,tau_slow=1*(10**-9)
                                                                                                                                                                                                           S_squared_fast, S_squared_slow, tau_slow,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         multi_plotter.__init__(self,parameter_min,parameter_max,npar,
                                                                                                                                                                  tau_m,i,S_squared,self.tau_e,model
                                                                                ,self.tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.relax_parameter=self.relax_plot_list[j]
in range(self.field_values):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.plot_units=self.plot_units_list[j]
self.file_label=self.file_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      tau_fast)][j]])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      S_squared_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               (0,10**12))
  for i
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 237
                                                    229
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      239
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             \frac{5}{2}68
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                233
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model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      model, S_squared_fast, S_squared_slow, tau_slow
                                                                                                                                                                                                                                                                                                 def plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        tau_fast), NOE(tau_m,i,self.S_squared,tau_e,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_fast), R2(tau_m,i,self.S_squared,tau_e,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             tau_fast), R1(tau_m,i,self.S_squared,tau_e
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           S_squared_slow,tau_slow,tau_fast),1.0/R2(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.L_plot.append([i,[1.0/R1(tau_m,i,self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 S_squared_fast, S_squared_slow, tau_slow,
                                                                                                                                                                                                                                                                                                                                                                                 S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)): #plot 1 is
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                      S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      tau_m,i,self.S_squared,tau_e,model,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                        handler so call it to run plot 1 and plot 2 for a given instance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.S_squared in self.parameter_value_list:
                                                                                                                                                               {/Symbol t}_m'+'='+str(tau_m)+'
                                                                                                                           {/Symbol t}_e'+'='+str(tau_e)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              for i in range(self.field_values)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   tau_fast)][i]])
                                                                                                                                                                                                             self.varied_parameter='S_squared'
                                                                               self.parameter_unit_label=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.constant_2)
self.value=5 #arbitrary
                                                                                                                                                                  self.constant_2='
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                in range (5):
                                                                                                                           self.constant_1='
                                      self.symbol = |S^2|
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.position=10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for j
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243

245

247

 249

251

269

253

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=10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical", S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             field_values=26, tau_m=10**-8, S_squared=0.85, tau_e=50*(10**-12), model
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         def plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      "classical", S_squared_fast=1, S_squared_slow=1, tau_slow=1*(10**-9)
                                                                                                                                                                                                                                                                                                                              __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      bу
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         <u>ω</u>
                                                                                                                                                                                                                                                                                                                                                                                                                                                       multi_plotter.__init__ (self,parameter_min,parameter_max,npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.model='extended' # Extended Lipari-Szabo eq'n presented
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.constant_2=' {/Symbol t}_{slow}'+'='+str(tau_slow)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.constant_1='S^2_{slow}'+'='+str(S_squared_slow)+
                                                                                                                                                                                                                                                                                                                                                                                                             S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)):
                                                                             self.relax_parameter=self.relax_plot_list[j]
                                                                                                                    self.plot_units=self.plot_units_list[j]
self.file_label=self.file_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               {/Symbol t}_{m}'+'='+str(tau_m)+' s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                fast}'+'='+str(S_squared_fast)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.symbol='{/Symbol t}_{fast}'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.varied_parameter='tau_fast'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.parameter_unit_label=' s'
                                                                                                                                                                                                                                                                                       tau_fast_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.value=5 #arbitrary
                                 (0,10**12))
                                                                                                                                                                                                   self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                 263
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   275
          257
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  271
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          273
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tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                      self.L_plot.append([i,[1.0/R1(tau_m,i,S_squared
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 S_squared_slow,tau_slow,self.tau_fast)][j]])
                                                                                                                                                                                                                                                                                                                                                            ,tau_e,model,S_squared_fast,S_squared_slow,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared_slow,tau_slow,self.tau_fast),NOE(
   the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       S_squared_slow,tau_slow,self.tau_fast),R1(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           S_squared_slow,tau_slow,self.tau_fast),R2(
                                                                                                                                            self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                         tau_slow, self.tau_fast),1.0/R2(tau_m,i,
                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       \mathtt{self.name\_prefix=self.file\_namer[j]+str(random.randint}
                                                                                                                                                                                                              self.y_label="set ylabel '%s'" % self.y_label_list[j]
S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)): #plot
                                  given instance
                                                                                                                                                                                                                                              for self.tau_fast in self.parameter_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.relax_parameter=self.relax_plot_list[j]
                                                                                                                                                                                                                                                                                    for i in range(self.field_values)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.plot_units=self.plot_units_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.file_label=self.file_label_list[j]
                                           ಡ
                              plot 1 and plot 2 for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           class tau_slow_varied_plotter(multi_plotter):
                                                                                                                                                                                self.constant_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       (0,10**12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.plot_2()
                                handler so call it to run
                                                                                                        for j in range(5):
                                                                self.position=10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                289
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              283
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          287
                                                                                                                  277
                                                                                                                                                                                                                         279
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      285
                                                                                                                                                                                                                                                                                               281
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          271
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field_values=26, tau_m=10**-8, S_squared=0.85, tau_e=50*(10**-12), model=
                                      ,S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                  ,S_squared_fast=1,S_squared_slow=1,tau_slow=1*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                           + '8^2_{
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)): #plot 1 is
__init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       bу
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              <u>ω</u>
                                                                                                                    multi_plotter.__init__(self,parameter_min,parameter_max,npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.model='extended' # Extended Lipari-Szabo eq'n presented
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            handler so call it to run plot 1 and plot 2 for a given instance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.constant_2=' {/Symbol t}_{fast}'+'='+str(tau_fast)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.tau_slow in self.parameter_value_list:
                                    =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical"
                                                                         S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                           self.constant_1='S^2_{slow}'+'='+str(S_squared_slow)+
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               for i in range(self.field_values):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   {/Symbol t}_{m}'+'='+str(tau_m)+' s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   fast}'+'='+str(S_squared_fast)
                                                                                                                                                                                                                                                                                                                                                               self.symbol='{/Symbol t}_{slow}'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.varied_parameter='tau_slow'
                                                                                                                                                                                                                                                                                                                                                                                                        self.parameter_unit_label='s
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.constant_2)
                                                                                                                                                                                                                                  tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                      self.value=5 #arbitrary
                                                                                                                                                                                                                                                                             self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           in range (5):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.position=10
                                                                                                                                                                                                  "classical"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 for j
        291
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     307
                                                                                                                                                                                                                                                                                           293
                                                                                                                                                                                                                                                                                                                                                                         295
                                                                                                                                                                                                                                                                                                                                                                                                                                                       297
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       272^{\frac{66}{68}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 303
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                305
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field_values=26, tau_m=10**-8, S_squared=0.85, tau_e=50*(10**-12), model=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical", S_squared_fast=1,
self.L_plot.append([i,[1.0/R1(tau_m,i,S_squared
                                                                                                                                                                                                                            tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                  tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                       tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared_slow,self.tau_slow,tau_fast)][j]])
                                               ,tau_e,model,S_squared_fast,S_squared_slow,
                                                                                                                                                                             S_squared_slow,self.tau_slow,tau_fast),NOE(
                                                                                                                                                                                                                                                                     S_squared_slow, self.tau_slow,tau_fast),R1(
                                                                                                                                                                                                                                                                                                                                                            S_squared_slow, self.tau_slow,tau_fast),R2(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          "classical",S_squared_fast=1,S_squared_slow=1,tau_slow=1*(10**-9),
                                                                                     self.tau_slow,tau_fast),1.0/R2(tau_m,i
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                    S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     multi_plotter.__init__(self,parameter_min,parameter_max,npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.relax_parameter=self.relax_plot_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.plot_units=self.plot_units_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.file_label=self.file_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            S_squared_fast_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.symbol='S^2_{fast}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.value=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                (0,10**12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     317
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    273^{\stackrel{\text{eff}}{\text{e}}}
             309
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  319
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     311
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               315
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          321
```

```
self.L_plot.append([i,[1.0/R1(tau_m,i,S_squared
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.S_squared_fast,S_squared_slow,tau_slow,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_fast), NOE(tau_m,i,S_squared,tau_e,model
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_fast), R1(tau_m,i,S_squared,tau_e,model,
                                                                                                                                                                                                                                                                                                                                                                    plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.S_squared_fast,S_squared_slow,tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_fast), R2(tau_m,i,S_squared,tau_e,model,
                                                                                                                                                                                                                                                 Clore
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    S_squared_slow,tau_slow,tau_fast),1.0/R2(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        S_squared_fast, S_squared_slow, tau_slow,
                                                                                                                                                                                                                                                                                                                                                                                                                                              S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)): #plot 1 is
                                                                                                                                                                                                                                               bу
                                                                                                                                                                                                                                                                                                                                                                                                         S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
                                                                                                                     ω
-
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for self.S_squared_fast in self.parameter_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_m,i,S_squared,tau_e,model,self
                                                                                                                                                                                                                                         self.model='extended' # Extended Lipari-Szabo eq'n presented
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ,tau_e,model,self.S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        handler so call it to run plot 1 and plot 2 for a given instance
                                                                                                                     self.constant_2=' {/Symbol t}_{fast}'+'='+str(tau_fast)+'
                                       self.constant_1='S^2_{slow}'+'='+str(S_squared_slow)+
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            for i in range(self.field_values)
                                                                                                                                                          {/Symbol t}_{m}'+'='+str(tau_m)+' s'
                                                                          Symbol t}_{alow}'+'='+str(tau_slow)+'
                                                                                                                                                                                                   self.varied_parameter='S_squared_fast'
self.parameter_unit_label=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.constant_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for j in range(5):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.position=10
        323
                                                                                                                                 325
                                                                                                                                                                                                                                                                                                                                                                               329
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              \frac{5}{274}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              333
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             335
                                                                                                                                                                                                                                                       327
```

```
field_values=26,tau_m=10**-8,S_squared=0.85,tau_e=50*(10**-12),model=
                                                                                                                                                                                                                                                                                                                                                                                                                                                       =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical", S_squared_fast=1,
self.S_squared_fast,S_squared_slow,tau_slow,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Clore
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      "classical", S_squared_fast=1, S_squared_slow=1, tau_slow=1*(10**-9),
                                                                                                                                                                                                                                                                                                                                                                                                                   __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             # Extended Lipari-Szabo eq'n presented by
                                                                                                          self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 multi_plotter.__init__ (self, parameter_min, parameter_max, npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     ω
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.constant_2=' {/Symbol t}_{fast}'+'='+str(tau_fast)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared_slow=1, tau_slow=1*(10**-9), tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.constant_1='S^2_{fast}'+'='+str(S_squared_fast)+
                                                                                                                                                                                      self.relax_parameter=self.relax_plot_list[j]
                                                                                                                                                                                                                        self.plot_units=self.plot_units_list[j]
                                                                                                                                                                                                                                                             self.file_label=self.file_label_list[j]
                                tau_fast)][j]])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               {/Symbol t}_{m}'+'='+str(tau_m)+' s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Symbol t}_{alow}'+'='+str(tau_slow)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.varied_parameter='S_squared_slow'
                                                                                                                                                                                                                                                                                                                                                                               S_squared_slow_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.parameter_unit_label=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.symbol='S^2_{slow}'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.value=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.model='extended'
                                                                                                                                                (0,10**12))
                                                                                                                                                                                                                                                                                                     self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                 class
                                                                                   337
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 355
                                                                                                                                                                                                339
                                                                                                                                                                                                                                                                                                                                                  343
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             347
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      349
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   353
                                                                                                                                                                                                                                                                        341
                                                                                                                                                                                                                                                                                                                                                                                                                           345
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              351
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     275
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tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.L_plot.append([i,[1.0/R1(tau_m,i,S_squared
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.S_squared_slow,tau_slow,tau_fast)][j]])
def plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.S_squared_slow,tau_slow,tau_fast),NOE(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.S_squared_slow,tau_slow,tau_fast),R1(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.S_squared_slow,tau_slow,tau_fast),R2(
                                                                                                                                                                                                                                                                               self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared_slow,tau_slow,tau_fast),1.0/R2(
                                            S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                    self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                   for self.S_squared_slow in self.parameter_value_list:
                                                                                    S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)): #plot
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ,tau_e,model,S_squared_fast,self.
                                                                                                                                    given instance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.relax_parameter=self.relax_plot_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for i in range(self.field_values)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.plot_units=self.plot_units_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.file_label=self.file_label_list[j]
                                                                                                                                    handler so call it to run plot 1 and plot 2 for
                                                                                                                                                                                                                                                                                                                            self.constant_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             (0,10**12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.plot_2()
                                                                                                                                                                                                                                for j in range(5):
                                                                                                                                                                                   self.position=10
```

361

357

359

369

367

```
field_values=26,tau_m=10**-8,S_squared=0.85,tau_e=50*(10**-12),model=
                                                                           ,S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        '+'S^2_{slow}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             def plot_1(self,parameter_min,parameter_max,npar,field_values=26,tau_m=10**-8,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.model='extended' # Extended Lipari-Szabo eq'n presented by Clore
                                                                                                                                                                                                                               "classical", S_squared_fast=1, S_squared_slow=1, tau_slow=1*(10**-9),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.g=(self.symbol,self.relax_plot_list[j],self.constant_1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)): #plot 1 is
                                   __init__ (self,parameter_min,parameter_max,npar,field_values=26,tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 S_squared=0.85,tau_e=50*(10**-12),model="classical",S_squared_fast=1,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        <u>ω</u>
                                                                                                                                                     nulti_plotter.__init__ (self,parameter_min,parameter_max,npar,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.y_label="set ylabel '%s'" % self.y_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 given instance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    {/Symbol t}_{fast}'+'='+str(tau_fast)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.constant_1='S^2_{fast}'+'='+str(S_squared_fast)+ '
                                                                       =10**-8, S_squared=0.85, tau_e=50*(10**-12), model="classical"
                                                                                                               S_squared_slow=1,tau_slow=1*(10**-9),tau_fast=1*(10**-12)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.parameter_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          {/Symbol t}_{slow}'+'='+str(tau_slow)+'s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             handler so call it to run plot 1 and plot 2 for
tau_m_extended_varied_plotter(multi_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                    self.parameter_unit_label='s'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.varied_parameter='tau_m'
                                                                                                                                                                                                                                                                                                                                                                                            self.symbol='{/Symbol t}_{m}'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  '+'='+str(S_squared_slow)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.constant_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.tau_m in
                                                                                                                                                                                                                                                                         tau_fast=1*(10**-12))
                                                                                                                                                                                                                                                                                                                                                     self.value=5 #arbitrary
                                                                                                                                                                                                                                                                                                                 self.i=5 #arbitrary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.constant_2=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             in range (5):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.position=10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        et al.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 for j
 371 class
                                                                                                                                                                                                                                                                                                                                                                 375
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          \overset{\varepsilon}{277}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           387
                                                                                                                                                                 373
                                                                                                                                                                                                                                                                                                                                                                                                                                            377
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       383
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         385
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      381
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S_squared_slow,tau_slow,tau_fast),1.0/R2(self
                                                                                                                                                       .tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                                                                  tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                          tau_m,i,S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'T_2' or 'NOE'; 'R_1', 'R_2
                                                                                                                                                                                                                                                                                                               tau_m,i,S_squared,tau_e,model,S_squared_fast
                                                                                                                                                                                             ,S_squared_slow,tau_slow,tau_fast),NOE(self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          =26, model="classical", S_squared_fast=1, S_squared_slow=1, tau_fast=1, tau_slow
                                                                                                                                                                                                                                                                      S_squared_slow,tau_slow,tau_fast),R1(self.
                                                                                                                                                                                                                                                                                                                                                     S_squared_slow,tau_slow,tau_fast),R2(self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         __init__ (self,relaxation_type,y_label,tau_m,tau_e,S_squared,field_values
                                 	exttt{self.L-plot.append([i,[1.0/R1(self.tau_m,i,
                                                                                                                                                                                                                                                                                                                                                                                                                                S_squared_slow,tau_slow,tau_fast)][j]])
                                                                          S_squared,tau_e,model,S_squared_fast,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.name_prefix=self.file_namer[j]+str(random.randint
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              structuring than 'plotter'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.relax_parameter=self.relax_plot_list[j]
in range(self.field_values):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.plot_units=self.plot_units_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.file_label=self.file_label_list[j]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.relaxation_type=relaxation_type #'T_1'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.gp=Gnuplot.Gnuplot(persist=1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   implementation for the multiple curves above
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            class single_plotter: #much better use of
 for i
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                (0,10**12))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.plot_2()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #plotting component
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      399
         389
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      278^{\frac{66}{68}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       403
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           397
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     401
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 391
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                395
```

```
slow}='+str(tau_slow)+' s,',' S^2_{fast}='+str(S_squared_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  {/Symbol t}_{fast}'+'='+str(tau_fast)+ ' s,', ' {/Symbol t}_{
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      )+', '+' S^2_{slow}='+str(S_squared_slow)+', '+' {/Symbol t}
                                                                                                                                                                                                                                                                                                                      self.output_file='../relaxation_tmp/'+self.file_name.replace('ps','png
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                strength on %s \\n\\n(%s %s %s)"' % (self.relaxation_type,'
                                                                                                                                                                                                           "../relaxation_tmp/%s"' % self.file_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   strength on %s \\n\\n(%s %s %s)"' % (self.relaxation_type,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           {/Symbol t}_e='+str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       of magnetic field
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.graph_title='set title "Influence of magnetic field
                                                                                                                                                                                                                                                                                                                                                                                            (self
                                                                                                                                                                           self.file_name='%s_tmp_%d.ps' % (relaxation_type,os.getpid())
                                                                                                                                                                                                                                                                                                                                                                                          self.convert_input='/usr/bin/convert -rotate 90 %s %s' %
                                                                   strength (Tesla)"')
                                                                                                                                                                                                                                                                                    self.input_file='../relaxation_tmp/'+self.file_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       t}_m='+str(tau_m)+ 's,', '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.graph_title='set title "Influence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         s,'', S^2='+str(S_squared))
term postscript enhanced color')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.y_label='set ylabel "%s"' % y_label
                                                                     field
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            _m='+str(tau_m)+' s,')
                                     data style lines')
                                                                                                                                                                                                                                                                                                                                                                                                                             input_file, self.output_file)
                                                                   xlabel "Magnetic
                                                                                                                                                                                                                self.input_to_gp='set output
                                                                                                     [0:25]')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   if self.model=="classical":
                                                                                                                                                                                                                                                self.gp(self.input_to_gp)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.gp(self.graph_title)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.gp(self.y_label)
                                                                                                         self.gp('set xrange
                                                                                                                                           self.gp('set nokey')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       {/Symbol
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         tau_e)+'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.model=model
                                                                     self.gp('set
   self.gp('set
                                   self.gp('set
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                else:
```

279

419

423

421

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407

409

411

```
tau_e,self.model,self.S_squared_fast,self.S_squared_slow,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              tau_e,self.model,self.S_squared_fast,self.S_squared_slow,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     tau_e,self.model,self.S_squared_fast,self.S_squared_slow,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          tau_e,self.model,self.S_squared_fast,self.S_squared_slow,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_e,self.model,self.S_squared_fast,self.S_squared_slow,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.T2_list.append([i,1.0/R2(self.tau_m,i,self.S_squared,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self. T1_list.append([i,1.0/R1(self.tau_m,i,self.S_squared,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.NOE_list.append([i,NOE(self.tau_m,i,self.S_squared,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.R1_list.append([i,R1(self.tau_m,i,self.S_squared,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.R2_list.append([i,R2(self.tau_m,i,self.S_squared,self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              .tau_fast,self.tau_slow)])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   . tau_fast, self.tau_slow)])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_fast, self.tau_slow)])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        tau_fast, self.tau_slow)])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_fast, self.tau_slow)])
                                                                                                                      self.S_squared_fast=S_squared_fast
                                                                                                                                                             self.S_squared_slow=S_squared_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         in range(field_values):
                                         squared
                                                                                                                                                                                                    self.tau_fast=tau_fast
                                                                                                                                                                                                                                           self.tau_slow=tau_slow
                                      self.S_squared=S_
self.tau_m=tau_m
                                                                             self.tau_e=tau_e
                                                                                                                                                                                                                                                                                                                                                                  \mathtt{self.NOE\_list=[]}
                                                                                                                                                                                                                                                                                  self.T1\_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                           self.R1_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                self.R2_list=[]
                                                                                                                                                                                                                                                                                                                           self.T2\_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    443
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427

429

433

431

435

437

280

439

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file</a></div>''' % (self.formatting, self.file_string.replace('../',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     print '''<div %s><h4>Additional data</h4><a href="%s">%s: CSV plot data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                f.write(str(coordinate).replace('[','').replace(']','')+'\n')
                                                                                                                                                                                                                                                                                                                                         Or
                                                                                                                                                                                                                                                                                                                                    be "T_1","T_2", "R_1","R_2"
                                                                                                                                                                                                                                                                                                                                                                                          self.file_string='../relaxation_tmp/%stext_out%d.csv' % (self
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.first_line='Field strength (Tesla),%s %s\n' % (self.
                                                                                                                                                                                                                                                                                                                                     print 'relaxation_type must
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     relaxation_type, self.plot_units)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       for coordinate in self.print_list:
                                                                                                                                                                                                                        self.plot_units = (s^{-1})'
                                                                                                                                                                                                                                                                             self.plot_units='(s^{-1})'
                                                                                   self.relaxation_type=='T_2':
                                                                                                                                                                                                                                                    self.relaxation_type=='R_2':
                                                                                                                                        self.relaxation_type=='NOE':
                                                                                                                                                                                               self.relaxation_type=='R_1':
                              if self.relaxation_type=='T_1':
                                                                                                                                                                                                                                                                                                                                                                                                                     relaxation_type,os.getpid())
                                                                                                           self.plot_units='(s)'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               '../../'), self.file_label)
                                                       self.plot_units='(s)'
                                                                                                                                                                                                                                                                                                                                                                                                                                               f=open(self.file_string,'w')
                                                                                                                                                                  self.plot_units=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    T1_single_plotter(single_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  f.write(self.first_line)
csv_printer(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           f.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           plot(self):
                                                                                                                                                                                                                                                                                                            else:
                                                                                                                                                                                                                                                    elif
                                                                                                                                                                                               elif
                                                                                                                                        elif
    def
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           467
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                                     445
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self.formatting='class="extra_data" style="top:1050px"'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.formatting='class="extra_data" style="top:526px"'
                                                                                                                                                                                                                                                                                                                                                                                                                                          self.file_label='<em>T<sub>2</sub></em>'
                                                                                                                                     self.file_label='<em>T<sub>1</sub></em>'
                                                                                                                                                              self.formatting='class="extra_data"'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  single_plotter.csv_printer(self)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        single_plotter.csv_printer(self)
                                                                                                                                                                                           single_plotter.csv_printer(self)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  self.file_label='<em>NOE</em>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.print_list=self.NOE_list
                                                    os.system(self.convert_input)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  os.system(self.convert_input)
                                                                                                                                                                                                                                                                                                                                                            os.system(self.convert_input)
                                                                                                         self.print_list=self.T1_list
                                                                                                                                                                                                                                                                                                                                                                                                                self.print_list=self.T2_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.gp.plot(self.NOE_list)
self.gp.plot(self.T1_list)
                                                                                                                                                                                                                                                                                                     self.gp.plot(self.T2_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NOE_single_plotter(single_plotter):
                                                                                                                                                                                                                                                T2_single_plotter(single_plotter):
                                                                                                                                                                                                                                                                                                                                                                                     csv_printer(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            csv_printer(self):
                                                                               csv_printer(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      time.sleep(1)
                                                                                                                                                                                                                                                                                                                                 time.sleep(1)
                          {	t time.sleep}\left(1
ight)
                                                                                                                                                                                                                                                                         def plot(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 def plot(self):
                                                                                                                                                                                                                                                                                                                                                                                        def
                                                                                                                                                                                                                                                 class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             489
     469
                                                                                                                                                                                                                                                                                                                                                                                        \overset{\tilde{s}}{\overset{8}{2}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    497
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                                                           471
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self.formatting='class="extra_data" style="top:1581px"'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.formatting='class="extra_data" style="top:2102px"'
                                                                                                                                                                                            self.file_label='<em>R<sub>1</sub></em>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.file_label='<em>R<sub>2</sub></em>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        single_plotter.csv_printer(self)
                                                                                                                                                                                                                                            single_plotter.csv_printer(self)
                                                                                                                    os.system(self.convert_input)
                                                                                                                                                                                                                                                                                                                                                                                                os.system(self.convert_input)
                                                                                                                                                                                                                                                                                                                                                                                                                                               self.print_list=self.R2_list
                                                                                                                                                                    self.print_list=self.R1_list
                                                                self.gp.plot(self.R1_list)
                                                                                                                                                                                                                                                                                                                                            self.gp.plot(self.R2_list)
                                                                                                                                                                                                                                                                                             R2_single_plotter(single_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   __init__ (self,cgi_p_varied):
                 R1_single_plotter(single_plotter):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #self.a=cgi_user_param
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.b=cgi_p_varied
                                                                                                                                         csv_printer(self):
                                                                                                                                                                                                                                                                                                                                                                                                                       def csv_printer(self):
                                                                                                                                                                                                                                                                                                                                                                      time.sleep(1)
                                                                                           time.sleep(1)
                                         def plot(self):
                                                                                                                                                                                                                                                                                                                     def plot(self):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          class frame2_generator:
                  class
                                                                                                                                                                                                                                                                                               class
                                                                                                                                                                                                                                                                                                  511
                                                                                                                                                                                                                                                                                                                                                283
499
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         527
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```
tau_slow':'3','S_squared_fast':'0.85','S_squared_slow':'0.77'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     partial order parameter','S_squared_slow':'slow partial order
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              tau_slow_low','S_squared_fast':'SS_fast_low','S_squared_slow'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          fast </ sub > ', 'S_squared_slow': 'S < sup > 2 < / sup > < sub > slow < / sub > ', '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.parameter_book={'tau_fast':'&tau<sub>fast</sub>','tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         ':'&tau<sub>slow</sub>','S_squared_fast':'S<sup>2</sub>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              parameter','tau_m_extended':'overall rotational correlation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.constant_dictionary={'tau_m':'10','tau_e':'50','S_squared
self.relax_book={'T1':'<em>T<sub>1</sub></em>','T2':'<em>T<sub>2</sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.descriptor_book={'tau_m':'overall rotational correlation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    tau_slow':'slow correlation time','S_squared_fast':'fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        time','
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.low_name_book={'tau_fast':'tau_fast_low','tau_slow':'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          time','
                                                                                                                                                                                                                                                                                                                                      self.constant_dictionary={'tau_m':'10','tau_fast':'10','
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            :'SS_slow_low','tau_m_extended':'tau_m_extended_low'}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.descriptor_book={'tau_fast':'fast correlation
                                                #dictionary corresponding
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        correlation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 S_squared':'generalized order parameter'}
                                                                                                                                                                                                                                                                                        self.b not in ['tau_m','tau_e','S_squared']:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        tau_m_extended':'&tau<sub>m</sub>'}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     times','tau_e':'effective internal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.p=self.parameter_book[self.b]
                                                                                                                                                                                                                                                                                                                                                                                                                                   ,'tau_m_extended':'10'}
                                              ></em>','NOE':'<em>NOE</em>'}
                                                                                                   relax type
                                                                                                                                                                                                                                              #self.r=self.relax_book[self.a]
                                                                                                 radio selection of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      : '0.85'}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               time'}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            else:
```

531

533

537

```
field
                                                                                                                                           self.parameter_book={'tau_m':'&tau<sub>m</sub>','tau_e':'&tau<
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  or 10 or 100; the default low value in the input
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             self.html_part1='''<html><head><link rel="stylesheet" type="text/css"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            value="ns" checked="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ''' % (self.p,self.b)
self.low_name_book={'tau_m':'taum_low','tau_e':'taue_low',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           trends with
                                                                                                                                                                                                                       sub>e</sub>','S_squared':'S<sup>2</sup>'} #dictionary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        value="ps"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #ps<input type="radio" name="tau_m_input_units2" value="ps"/>
                                                                                                                                                                                                                                                                                              corresponding to second radio selection of params
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               name="tau_m_input_units1" value="s"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           on relaxation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        name="tau_m_input_units1"
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                                                                                                                                                                                                                                                                                                                                                                              self.p=self.parameter_book[self.b]
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    /></head>
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,&nbsp %s''' % (self.descriptor_book[self.b],self.p) + '''<</pre>
                                                                                                                                                                                                                                                                                                                           ,'_high'),self.high_value) + self.radio_high + '''
                                                                                                                                                                                                                                                                                             s" value="%s">''' % (self.p, self.low_name_book[self.b].replace('_low'
                                                                                                                   values for the %s
                                                                                                                                                                                                                                                                  ''' + '''Upper bound for %s --> <input type="text" name="%"</pre>
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                                                                                                                                                                                                                                                                                                                                                                                       %s (0-14)
                                                                                                                                                                                                        --> <input type="text" name="%s" value="%s">''' % (self.p,self.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #self.constant_dictionary={'tau_m':'10','tau_e':'50','S_squared
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                                                          name="tau_m_input_units2" value="s"/>
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                                                                                                                                                                            tr>Lower bound for %s
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self.parameter_book.items()[2][0]], self.parameter_book.items
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self.b not in ['tau_m','tau_e','S_squared']:
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name="%s_input" value="%s"> ''' % (self.parameter_book.items()
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[1][0]]) + self.constant_radio_2 + '''
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                       <input class="input_right" style="top:220px" type="submit"</pre>
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self.constant_radio_2='''ps<input type="radio" name="tau_m_input_units"
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<input class="input_right" style="top:165px" type="submit"</pre>
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                                                                                                                                                                                                                                                                        print self.html_part1+self.html_part2()+self.html_part3()
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                             value="plot">
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  frame2_generator.__init__(self,cgi_p_varied)
                                                        frame2_generator.__init__(self,cgi_p_varied)
                                                                                                                                               self.radio_low='''ps<input type="radio"
                                                                                       #low and high input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #low and high input and defaults
                                                                                                                                                                                                                                                                                                                                                                                                                                                          #constants input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_m_varied_input(frame2_generator):
tau_e_varied_input(frame2_generator):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.constant_radio_2='' #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     __init__ (self,cgi_p_varied):
                                                                                                                                                                               checked="yes"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ="ps" checked="yes"/>
                             __init__ (self,cgi_p_varied)
                                                                                                                                                                                                                                                                      self.high_value='50.0'
                                                                                                                                                                                                                                                                                                                                                                ns<input type="radio"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ns<input type="radio"
                                                                                                                    self.low_value='10.0'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.low_value='1'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      value="ps"/>
                                                                                                                                                                                                                                                                                                                                    value="ps"/>
                                                                                                                                                                                                               ns<input
class
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         class
                                                                                                                                                                                                                                                                                                                                                                                                                               290
                                    645
                                                                                               647
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checked="yes
                                                                                                                                                                                                                                                                               name="tau_e_input_units"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.radio_high='''ps<input type="radio" name="tau_fast_input_units2"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        self.radio_low='''ps<input type="radio" name="tau_fast_input_units1"
                                                                                            name="tau_m_input_units2"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        s<input type="radio" name="tau_fast_input_units2" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      name="tau_fast_input_units1" value="ns"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            type="radio" name="tau_fast_input_units2" value="ns"/>
                             value="s"/>'''
                                                                                                                                                                                                                 value="s"/>'''
ns<input type="radio" name="tau_m_input_units1" value="ns"/>
                                                                                                                                                                                                                                                                                                                                                                       s<input type="radio" name="tau_e_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                            ns<input type="radio" name="tau_e_input_units" value="ns"/>
                                                                                                                                                       value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                        no unit selection for S^2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    name="tau_fast_input_units1"
                                                                                                                                                       name="tau_m_input_units2"
                             s<input type="radio" name="tau_m_input_units1"
                                                                                                                                                                                                                                                                               self.constant_radio_1='''ps<input type="radio"
                                                                                                                                                                                                                 s<input type="radio" name="tau_m_input_units2"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  frame2_generator.__init__(self,cgi_p_varied)
                                                                                         type="radio"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #low and high input and defaults:
                                                                                                                                                                                                                                                  defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     tau_fast_varied_input(frame2_generator):
                                                                                                                                                                                                                                                                                                             checked="yes"/>
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                value="ps" checked="yes"/>
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                                                                                            self.radio_high='''ps<input
                                                                                                                                                                                                                                                                                                                                                                                                      self.constant_radio_2='' #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  __init__ (self,cgi_p_varied):
                                                           self.high_value='10.0'
                                                                                                                                                       ns<input type="radio"
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name="tau_m_input_units"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              checked="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            checked="
                                                   checked="yes
                                                                                                                                                                                                                                             checked="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.radio_high='''ps<input type="radio" name="tau_slow_input_units2"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.radio_low='''ps<input type="radio" name="tau_slow_input_units1"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               s<input type="radio" name="tau_slow_input_units2" value="s"/>'''
                                                                                                                                                                                                                                                                                              s<input type="radio" name="tau_slow_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             name="tau_slow_input_units1" value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         name="tau_slow_input_units2" value="ns"
                                                                                                                                                                                                                                         type="radio" name="tau_slow_input_units" value="ns"
                                                                                                       s<input type="radio" name="tau_m_input_units" value="s"/>'''
                                                   value="ns"
                                                                                                                                                                                     self.constant_radio_4='''ps<input type="radio" name="
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self.constant_radio_1='''ps<input type="radio"
                                                   name="tau_m_input_units"
                                                                                                                                                               selection
                                                                                                                                                                                                                                                                                                                                                                                                          frame2_generator.__init__(self,cgi_p_varied)
                                                                                                                                                                                                                value="ps"/>
                                                                                                                                    no unit
                                                                                                                                                              # no unit
                                                                                                                                                                                                                                                                                                                                                                                                                                  #low and high input and defaults:
                                                                                                                                                                                                                                                                                                                                                    tau_slow_varied_input(frame2_generator):
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                                                                                                                                                            self.constant_radio_3=''
                                                                                                                                  self.constant_radio_2=''
                                                                                                                                                                                                                  tau_slow_input_units"
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                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.low_value='3'
                         value="ps"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              value="ps"/>
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                                                                                                                                                                                                                                                                   yes"/>
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      693
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self.constant_radio_1='''ps<input type="radio" name="tau_m_input_units"
                                                         checked="yes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  name="tau_m_input_units
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             checked="yes
                                                                                                                                                                                                                                                                                               s<input type="radio" name="tau_fast_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                     ns<input type="radio" name="tau_fast_input_units" value="ns"/>
                                                                                                                  s<input type="radio" name="tau_m_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        s<input type="radio" name="tau_m_input_units" value="s"/>'''
                                                         name="tau_m_input_units" value="ns"
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                                                                                                                                                    slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     # no unit selection for S slow
                                                                                                                                                                                                          self.constant_radio_4='''ps<input type="radio" name="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.constant_radio_3='''ps<input type="radio" name="
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                                                                                                                                                                            Ø
                                                                                                                                                                                                                                        tau_fast_input_units" value="ps" checked="yes"/>
                                                                                                                                                                               for
                                                                                                                                                    selection for
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                                                                                                                                                                               selection
                                                                                                                                                                                                                                                                                                                                                                                                                      frame2_generator.__init__(self,cgi_p_varied)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    self.radio_high='' # S fast has no units
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               fast has no units
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_slow_input_units" value="ps"/>
                                                                                                                                                                                                                                                                                                                                                           S_squared_fast_varied_input(frame2_generator):
                                                                                                                                                 # no unit
                                                                                                                                                                             # no unit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.constant_radio_1='''ps<input
                                                                                                                                                                                                                                                                                                                                                                                                                                                 #low and high input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #constants input and defaults:
                                                                                                                                                                            self.constant_radio_3=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   self.constant_radio_2=''
                                                                                                                                                 self.constant_radio_2=''
                                                                                                                                                                                                                                                                                                                                                                                         __init__ (self,cgi_p_varied):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.radio_low='' # S
                                                         ns<input type="radio"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         self.high_value='1.0'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.low_value='0.0'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                value="ps"/>
                            value="ps"/>
                                                                                                                                                                                                                                                                                                                                                              class
      715
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         737
                                                                                                                           717
                                                                                                                                                                                                                                                                                                                                                                                                                         293<sup>5</sup>
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self.constant_radio_1='''ps<input type="radio" name="tau_m_input_units"
   checked="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     checked="yes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ns<input type="radio" name="tau_slow_input_units" value="ns" checked="
                                                                                                                                                                                         s<input type="radio" name="tau_fast_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            s<input type="radio" name="tau_slow_input_units" value="s"/>'''
                                                               s<input type="radio" name="tau_slow_input_units" value="s"/>'''
                                                                                                                                                             name="tau_fast_input_units" value="ns"/>
 name="tau_slow_input_units" value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                s<input type="radio" name="tau_m_input_units" value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  # no unit selection for S fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                self.constant_radio_3='''ps<input type="radio" name="
                                                                                              self.constant_radio_4='''ps<input type="radio" name="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            self.constant_radio_4='''ps<input type="radio" name="
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          au_fast_input_units" value="ps" checked="yes"/>
                                                                                                                             value="ps" checked="yes"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   name="tau_m_input_units"
                                                                                                                                                                                                                                                                                                                           frame2_generator.__init__(self,cgi_p_varied)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        units
                                                                                                                                                                                                                                                                                                                                                                                                                          units
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              tau_slow_input_units" value="ps"/>
                                                                                                                                                                                                                                                           S_squared_slow_varied_input(frame2_generator):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          no
                                                                                                                                                                                                                                                                                                                                                                                                                              no
                                                                                                                                                                                                                                                                                                                                                      #low and high input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     self.radio_high='' # S slow has
                                                                                                                                                                                                                                                                                                                                                                                                                         slow has
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #constants input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              self.constant_radio_2=''
                                                                                                                             tau_fast_input_units"
                                                                                                                                                                                                                                                                                          __init__ (self,cgi_p_varied):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ns<input type="radio"
type="radio"
                                                                                                                                                             ns<input type="radio"
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                                                                                                                                                                                                                                                                                                                                                                                                                                                      self.high_value='1.0'
                                                                                                                                                                                                                                                                                                                                                                                        self.low_value='0.0'
                                                                                                                                                                                                                                                                                                                                                                                                                     self.radio_low='' #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     value="ps"/>
   ns<input
                                                                                                                                                                                                                                                             class
                                                                         739
                                                                                                                                                                                                                                                                                                                                                                                                                                                        294<sup>52</sup>
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type="radio" name="tau_slow_input_units" value="ns" checked="
                                                                                                                                                                                                                                                                                                                                                                                                                                           value="s"/>'''
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                                                                                                                                                                                                                                                                                                                                                                          value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ns<input type="radio" name="tau_m_extended_input_units2" value="ns"
                                value="s"/>'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     s<input type="radio" name="tau_slow_input_units" value="s"/>'''
ns<input type="radio" name="tau_fast_input_units" value="ns"/>
                                                                                                                                                                                                                                                                                                                                                                        ns<input type="radio" name="tau_m_extended_input_units1"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              s<input type="radio" name="tau_m_extended_input_units2"
                                                                                                                                                                                                                                                                                                                                                                                                                                           s<input type="radio" name="tau_m_extended_input_units1"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   slow
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       self.constant_radio_4='''ps<input type="radio" name="
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     au_fast_input_units" value="ps" checked="yes"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   selection for
                                s<input type="radio" name="tau_fast_input_units"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   # no unit selection for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          self.radio_high='''ps<input type="radio" name="
                                                                                                                                                                                                                                                                                                      self.radio_low='''ps<input type="radio" name="
                                                                                                                                                                                                       frame2_generator.__init__(self,cgi_p_varied)
                                                                                                                                                                                                                                                                                                                                     tau_m_extended_input_units1" value="ps"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_m_extended_input_units2" value="ps"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_slow_input_units" value="ps"/>
                                                                                                                                      tau_m_extended_varied_input(frame2_generator):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   # no unit
                                                                                                                                                                                                                                     #low and high input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #constants input and defaults:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               self.constant_radio_2=''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 self.constant_radio_1=''
                                                                                                                                                                      __init__ (self,cgi_p_varied):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           self.high_value='40.0'
                                                                                                                                                                                                                                                                       self.low_value='1.0'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              checked="yes"/>
                                                                                                                                                                                                                                                                                                                                                                                                         checked="yes"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ns<input
                                                                                                                                      class
                                                                                                                                           765
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295
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                                                                          763
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```

Listing C.4: CGI/html printing module: multi_curve.py

```
print '<head><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     form=cgi.FieldStorage() #retrieves a dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          instance=class_storage.SS_varied_input(b)
                                                                                                                                                                                                                                                                                                                                                                                                                            ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 instance.print_html()
                                                                                                                                                                                                                                                                                    print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                                                                       os.environ['PATH']=os.pathsep.join\
                                                                                                                  import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    b=form['p_varied'].value
                                                                                                                                            from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                                                                                                                                                                      #os.putenv('DISPLAY',':10.0')
                                                                    cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    if b == 'S_squared':
                                                                                                                                                                                                                                                                                                                                                          main.css" /></head>'
                                                                                                                                                                  import class_storage
                                                                                            math
                                             cgi
                                                                                                                                                                                           import os
                      import
                                                                      import
                                                                                              import
                                             import
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                try:
                                                                                                                                                                                                                                                                                          គ្
297
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```

```
fields
                                                                   content="0; url=/%s_varied_%s.html
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            a11
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print ' Please fill
                                                                                                                                                                                                                                                                                                                                                                                                                              instance=class_storage.S_squared_slow_varied_input(b)
                                                                                                                                                                                                                                                                                                                                                            instance=class_storage.S_squared_fast_varied_input(b)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      instance=class_storage.tau_m_extended_varied_input(b)
                                                                                                                                                                                                               instance=class_storage.tau_fast_varied_input(b)
                                                                                                                                                                                                                                                                                     instance=class_storage.tau_slow_varied_input(b)
                     instance=class_storage.tau_e_varied_input(b)
                                                                                                                                          instance=class_storage.tau_m_varied_input(b)
                                                                   #print '<meta http-equiv="refresh"
                                            instance.print_html()
                                                                                                                                                                instance.print_html()
                                                                                                                                                                                                                                      instance.print_html()
                                                                                                                                                                                                                                                                                                           instance.print_html()
                                                                                                                                                                                                                                                                                                                                                                                 instance.print_html()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            instance.print_html()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             b=='tau_m_extended':
                                                                                                                                                                                                                                                                                                                                                                                                        b== 'S_squared_slow':
                                                                                                                                                                                                                                                                                                                                 b=='S_squared_fast':
                                                                                          % (b,a)
                                                                                                                                                                                                                                                               b=='tau_slow':
                                                                                                                                                                                         b=='tau_fast':
                                                                                                                  b=='tau_m':
b=='tau_e':
                                                                                          1 </ |
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for step
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          sys.exit()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      except:
                           29
                                                                                                                                                                                                                                                                                         39
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                                                                                                                                                                                                                                                                                                                                     298
```

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          elif form['tau_fast_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      if form['tau_fast_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               tau_fast=float(form['tau_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                 ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_fast=tau_fast*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_fast=tau_fast*(10**-9)
                                                                                                                                                                                                                                                                                                       print 'Content-Type: text/html\n\n'
                                                                                                                                                                                                                                                                                                                                                                                                       os.environ['PATH']=os.pathsep.join\
                                                                                                                                                  import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                              from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                                 cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                  main.css" /></header>'
                                                                                                                                                                                                     import class_storage
                                                                                                                           math
                                                                                                                                                                                                                                  0
                                                                                                    import
                                                  import
                                                                          import
                                                                                                                            import
                                                                                                                                                                                                                                import
                                                                                                                                                                                                                                                                                                                                                                                                                                                               try:
                                                                                                                                                                                                                                                                                                                                                                                                                16
                                                                                                                                 9
                                                                                                                                                                                                                                                                                     299
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      24
```

```
value), npar=int(form['npar'].value),field_values=26,model="extended",tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    instance_1=class_storage.S_squared_fast_varied_plotter(tau_m=w,parameter_min=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              float(form['SS_fast_low'].value),parameter_max=float(form['SS_fast_high'].
                                                                                                                                                                                                                                                                                                                                                                                                                               tau_m=float(form['tau_m_extended_input'].value)
                                                                                                                                                                                               elif form['tau_slow_input_units'].value=='ps':
                                                                                                                                        if form['tau_slow_input_units'].value=='ns':
                                                                                                            tau_slow=float(form['tau_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                          b=float(form['S_squared_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                           if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                            tau_slow=tau_slow*(10**-12)
                                                                                                                                                                     tau_slow=tau_slow*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       =a,S_squared_slow=b,tau_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          print form [key]. value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               tau_m=tau_m*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_m = tau_m * (10 * * -9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #for key in form.keys():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #print form.keys()
                                                                                                                                                                                                                                                                                      pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            pass
                                                                                                                                                                                                                                                                                                                 a=tau_slow
                                                       c=tau_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      W = tau_m
                                                                                                                                                                                                                                                          else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             else:
else:
                                 28
                                                                                         30
                                                                                                                                                32
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                                                                                                                                                                                                                                                                                                                       38
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               84
                                                                                                                                                                                                                                                                                                                                                                                                       300
```

54	<pre>instance_1.plot_1(tau_m=w,parameter_min=float(form['SS_fast_low'].value), parameter_max=float(form['SS_fast_high'].value),npar=int(form['npar'].value), field_values=26,model="extended",tau_slow=a,S_squared_slow=b,tau_fast=c)</pre>	
except:		
δυ 00	print *center/Flease ensure all input values are number characters/center/ sys.exit()	

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      a dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_m=float(form['tau_m_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         tau_m = tau_m * (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        tau_m=tau_m*(10**-9)
                                                                                                                                                                                                                                                                                                          print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                                                                                                      os.environ['PATH']=os.pathsep.join\
                                                                                                                                                    import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                                 from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                                                                                                                                                                                       #os.putenv('DISPLAY',':10.0')
                                                                                                   cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                       main.css" /></header>'
                                                                                                                                                                                                        import class_storage
                                                                                                                             math
                                                                                                     import
                                                  import
                                                                                                                              import
                                                                                                                                                                                                                                    import
                                                                           import
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                try:
                                                                                                                                   9
                                                                                                                                                                                                                                      10
                                                                                                                                                                                                                                                                                         302
                                                                                                                                                                                                                                                                                                                                            14
                                                                                                                                                                                                                                                                                                                                                                                                                        16
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  26
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              22
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                24
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           20
```

```
(form['SS_high'].value),npar=int(form['npar'].value),field_values=26,tau_m=a,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             instance_1.plot_1(parameter_min=float(form['SS_low'].value),parameter_max=float
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   print '<center>Please ensure all input values are number characters</center>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                              SS_low'].value),parameter_max=float(form['SS_high'].value),npar=int(form['
                                                                                                                                                                                                                                                                                                                                                                                                                                    instance_1=class_storage.S_squared_varied_plotter(parameter_min=float(form['
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               npar'].value),field_values=26,tau_m=a,tau_e=b)
                                                                                                                                                                                                              elif form['tau_e_input_units'].value=='ps':
                                                                                                                                                   if form['tau_e_input_units'].value=='ns':
                                                                                                                     tau_e=float(form['tau_e_input'].value)
                                                                                                                                                                                                                                              tau_e=tau_e*(10**-12)
                                                                                                                                                                                 tau_e=tau_e*(10**-9)
                                                                                                                                                                                                                                                                                                           pass
                             pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    sys.exit()
                                                             a=tau_m
                                                                                                                                                                                                                                                                                                                                            b=tau_e
else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          except:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               44
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            46
                                    28
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                                                                                                                                                            32
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                                                                                                                                                                                                                                                                                     36
                                                                                                                                                                                                                                                                                                                                                38
                                                                                                                                                                                                                                                                                                                                                                                                              40
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        42
                                                                                                                                                                                                                                                                                                                                                                                                                                       303
```

Listing C.7: Module (plot_S_squared_slow.py) to plot input data by calling appropri-

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           elif form['tau_fast_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         if form['tau_fast_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   tau_fast=float(form['tau_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                        ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   tau_fast=tau_fast*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_fast=tau_fast*(10**-9)
                                                                                                                                                                                                                                                                                                print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                                                              os.environ['PATH']=os.pathsep.join\
                                                                                                                                               import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                          from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                               cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                         main.css" /></header>'
                                                                                                                                                                                                 import class_storage
                                                                                                                        math
                                                                        cgi
                                                                                                                                                                                                                            0
                                                                                                 import
                                                import
                                                                        import
                                                                                                                         import
                                                                                                                                                                                                                           import
                                                                                                                                                                                                                                                                                                                                                                                                                                                    try:
                                                                                                                              9
                                                                                                                                                                                                                                                                               304
                                                                                                                                                                                                                                                                                                                                                                                                       16
                                                                                                                                                                                                                               10
                                                                                                                                                                                                                                                                                                                                14
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         26
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         22
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         24
```

```
value), npar=int(form['npar'].value),field_values=26,model="extended",tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    instance_1=class_storage.S_squared_slow_varied_plotter(tau_m=w,parameter_min=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              float(form['SS_slow_low'].value),parameter_max=float(form['SS_slow_high'].
                                                                                                                                                                                                                                                                                                                                                                                                                               tau_m=float(form['tau_m_extended_input'].value)
                                                                                                                                                                                               elif form['tau_slow_input_units'].value=='ps':
                                                                                                                                        if form['tau_slow_input_units'].value=='ns':
                                                                                                            tau_slow=float(form['tau_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                          b=float(form['S_squared_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                           if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                            tau_slow=tau_slow*(10**-12)
                                                                                                                                                                     tau_slow=tau_slow*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       =a,S_squared_fast=b,tau_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          print form [key]. value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               tau_m=tau_m*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_m = tau_m * (10 * * -9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #for key in form.keys():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #print form.keys()
                                                                                                                                                                                                                                                                                      pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            pass
                                                                                                                                                                                                                                                                                                                 a=tau_slow
                                                       c=tau_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      W = tau_m
                                                                                                                                                                                                                                                          else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             else:
else:
                                 28
                                                                                         30
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ^{52}
                                                                                                                                                                                                                                                                                                                       38
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               84
                                                                                                                                                                                                                                                                                                                                                                                                       305
```

```
parameter_max=float(form['SS_slow_high'].value),npar=int(form['npar'].value),
                                                                                                                                                                                                  number characters</center>'
                                                                           field_values=26,model="extended",tau_slow=a,S_squared_fast=b,tau_fast=c)
instance_1.plot_1(tau_m=w,parameter_min=float(form['SS_slow_low'].value),
                                                                                                                                                                                                print '<center>Please ensure all input values are
                                                                                                                                                                                                                                     sys.exit()
                                                                                                                                                              except:
                                                                                                                                                                    26
         54
                                                                                                                                                                                                                                                 22
```

Listing C.8: Module (plot_tau_e.py) to plot input data by calling appropriate class

```
([os.environ['PATH'],'/home/structbi/Tyler_programs/gnuplot-4.2.5/src/gnuplot'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                           #os.system("alias gnuplot='/home/structbi/Tyler_programs/gnuplot-4.2.5/src/gnuplot'")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #print os.popen('which gnuplot').readlines()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     os.environ['PATH']=os.pathsep.join\
                                                                                                                                                                                                                                                                                                                                                                                    print 'Content-Type: text/html/n/n'
                                                                                                                                                                           import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                                                         from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                                                                                                                                                                                                                                                                                                                                                                             #os.putenv('DISPLAY',':10.0')
                                                                                                                 import cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              main.css" /></header>'
                                                                                                                                                                                                                                    import class_storage
                                                                                                                                               math
                                                             sys
                                                                                                                                                                                                                                                                    import os
                                                          import
                                                                                                                                               import
                                                                                     import
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                                                                                                                                                                                                                                                                                                                                 307
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```

```
form=cgi.FieldStorage() #retrieves a dictionary with form info
                                                                                                   elif form['tau_e_input_units1'].value=='ps':
    k=(10**-12)
                                                                                                                                                                                                                                                  elif form['tau_e_input_units2'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                       elif form['tau_m_input_units'].value=='ps':
                                                           if form['tau_e_input_units1'].value=='ns':
                                                                                                                                                                                                            if form['tau_e_input_units2'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                            if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               b=float(form['S_squared_input'].value)
                                                                                                                                                                                                                                                                                                                                                           tau_m=float(form['tau_m_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_m = tau_m * (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                   tau_m=tau_m*(10**-9)
                                                                                                                                                                                                                                                                      j = (10**-12)
                                                                                                                                                                                                                             j = (10**-9)
                                                                                 k = (10**-9)
                                                                                                                                                                                                                                                                                                                  j=1
                                                                                                                                                                                                                                                                                               else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   else:
                                                                                                                                                else:
26 try:
                                                                                                                                                                                                                                                                                               308
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         22
```

```
taue_low'].value)*k,parameter_max=float(form['taue_high'].value)*j,npar=int(
                                                                                                                                           instance_1.plot_1(parameter_min=float(form['taue_low'].value)*k,parameter_max=
                                                                                                                                                                                                                                                                                                                                                                                         print '<center>Please ensure all input values are number characters</center>'
                                                                                                                                                                                          float(form['taue_high'].value)*j,npar=int(form['npar'].value),field_values
instance_1=class_storage.tau_e_varied_plotter(parameter_min=float(form['
                                                                                               form['npar'].value),field_values=26,tau_m=a,S_squared=b)
                                                                                                                                                                                                                                                =26, tau_m=a, S_squared=b)
                                                                                                                                                                                                                                                                                                                                                                                                                                             sys.exit()
                                                                                                                                                                                                                                                                                                                                                    except:
              26
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```

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            elif form['tau_fast_input_units1'].value=='ps'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if form['tau_fast_input_units1'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                  ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                  ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                 form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                      print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                            os.environ['PATH']=os.pathsep.join\
                                                                                                                                   import Gnuplot, Gnuplot.funcutils
                                                                                                                                                           from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                      cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    k = (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        k = (10**-9)
                                                                                                                                                                                                                                                                                                                                        main.css" /></header>'
                                                                                                                                                                               import class_storage
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           else:
                                                                                                             math
                                                                  cgi
                                                                                                                                                                                                        0
                                                                                       import
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                                                                                                              import
                                                                                                                                                                                                                                                                                                                                                                                                             try:
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```

```
tau_m=float(form['tau_m_extended_input'].value)
                                                                                                                  elif form['tau_fast_input_units2'].value=='ps'
                                                                                                                                                                                                                                                                                                                                                                   elif form['tau_slow_input_units'].value=='ps':
                                                       if form['tau_fast_input_units2'].value=='ns':
                                                                                                                                                                                                                                                                                                      if form['tau_slow_input_units'].value=='ns':
                                                                                                                                                                                                                                                                         tau_slow=float(form['tau_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     b=float(form['S_squared_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      c=float(form['S_squared_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                tau_slow=tau_slow*(10**-12)
                                                                                                                                                                                                                                                                                                                                    tau_slow=tau_slow*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print form [key]. value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       tau_m=tau_m*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           tau_m=tau_m*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #for key in form.keys():
                                                                                                                                               j = (10**-12)
                                                                                       j = (10**-9)
k=1
                                                                                                                                                                                                                j=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         a=tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     W = tau_m
                                                                                                                                                                                    else:
                                                                                                                                                                                                                                                                                                                                                                                                                                else:
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```
form['tau_fast_low'].value)*k,parameter_max=float(form['tau_fast_high'].value
                                                                                                                               )*j,npar=int(form['npar'].value),field_values=26,model="extended",tau_slow=a,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   input values are number characters</center>'
                                         instance_1=class_storage.tau_fast_varied_plotter(tau_m=w,parameter_min=float(
                                                                                                                                                                                                                                 instance_1.plot_1(tau_m=w,parameter_min=float(form['tau_fast_low'].value)*k
                                                                                                                                                                                                                                                                                parameter_max=float(form['tau_fast_high'].value)*j,npar=int(form['npar']
                                                                                                                                                                                                                                                                                                                              value), field_values=26, model="extended", tau_slow=a, S_squared_slow=b,
                                                                                                                                                                                   S_squared_slow=b,S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         print '<center>Please ensure all
                                                                                                                                                                                                                                                                                                                                                                               S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               sys.exit()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      except:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           61
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        63
```

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                    a dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     elif form['tau_m_extended_input_units1'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        if form['tau_m_extended_input_units1'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                    ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                  form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                  print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                                            os.environ['PATH']=os.pathsep.join\
                                                                                                                                         import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                  from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                          cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              k = (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                k = (10**-9)
                                                                                                                                                                                                                                                                                                                                                        main.css" /></header>'
                                                                                                                                                                                        import class_storage
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      else:
                                                                                                                  math
                                                                     cgi
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                                                                                             import
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                                                                                                                                                                                                                                                                                                                                                                                                                               try:
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   25
```

```
elif form['tau_m_extended_input_units2'].value=='ps':
                                                          if form['tau_m_extended_input_units2'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                         elif form['tau_slow_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     elif form['tau_fast_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                            if form['tau_slow_input_units'].value=='ns':
                                                                                                                                                                                                                                                                              tau_slow=float(form['tau_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            tau_fast=float(form['tau_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          if form['tau_fast_input_units'].value=='ns'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               b=float(form['S_squared_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                c=float(form['S_squared_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                         tau_slow=tau_slow*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     tau_fast=tau_fast*(10**-12)
                                                                                                                                                                                                                                                                                                                                            tau_slow=tau_slow*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         tau_fast=tau_fast*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            print form [key]. value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #for key in form.keys():
                                                                                                                                                  j = (10**-12)
                                                                                        j = (10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           pass
k=1
                                                                                                                                                                                                                    j=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   a=tau_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   w=tau_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      else:
                                                                                                                                                                                        else:
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```
instance_1=class_storage.tau_m_extended_varied_plotter(tau_fast=w,parameter_min
                                                                                                                                  tau_m_extended_high'].value)*j,npar=int(form['npar'].value),field_values=26,
                                                                                                                                                                                                                                                                               value)*k,parameter_max=float(form['tau_m_extended_high'].value)*j,npar=int(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     number characters</center>'
                                                                                                                                                                                                                                  instance_1.plot_1(tau_fast=w,parameter_min=float(form['tau_m_extended_low']
                                                                                   =float(form['tau_m_extended_low'].value)*k,parameter_max=float(form['
                                                                                                                                                                                                                                                                                                                                form['npar'].value),field_values=26,model="extended",tau_slow=a,
                                                                                                                                                                                    model="extended",tau_slow=a,S_squared_slow=b,S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     input values are
                                                                                                                                                                                                                                                                                                                                                                                 S_squared_slow=b,S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print '<center>Please ensure all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  sys.exit()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         except:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              61
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```

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                                   a dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             elif form['tau_m_input_units1'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if form['tau_m_input_units1'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                   ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                   form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                  print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                                           os.environ['PATH']=os.pathsep.join\
                                                                                                                                         import Gnuplot, Gnuplot.funcutils
                                                                                                                                                                  from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                                                                                                                                                                  #os.putenv('DISPLAY',':10.0')
                                                                                          cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     k = (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        k = (10**-9)
                                                                                                                                                                                                                                                                                                                                                       main.css" /></header>'
                                                                                                                                                                                       import class_storage
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     k=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             else:
                                                                                                                  math
                                                                     cgi
                                                                                            import
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                                                                                                                                                                                                                                                                                                                                                                                                                              try:
                                                                                                                                                                                                                                                                   316
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```

```
taum_low'].value)*k,parameter_max=float(form['taum_high'].value)*j,npar=int(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    instance_1.plot_1(parameter_min=float(form['taum_low'].value)*k,parameter_max=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          print '<center>Please ensure all input values are number characters</center>'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    float(form['taum_high'].value)*j,npar=int(form['npar'].value),field_values
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    instance_1=class_storage.tau_m_varied_plotter(parameter_min=float(form['
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    form ['npar'].value),field_values=26,tau_e=a,S_squared=b)
                                                                                    elif form['tau_m_input_units2'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                            elif form['tau_e_input_units'].value=='ps':
                           if form['tau_m_input_units2'].value=='ns':
                                                                                                                                                                                                                                                                                             if form['tau_e_input_units'].value=='ns':
                                                                                                                                                                                                                                                               tau_e=float(form['tau_e_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   b=float(form['S_squared_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                              tau_e=tau_e*(10**-12)
                                                                                                                                                                                                                                                                                                                             tau_e=tau_e*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         =26,tau_e=a,S_squared=b)
                                                                                                                      j = (10**-12)
                                                       j = (10**-9)
                                                                                                                                                                                               j=1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             except:
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```

sys.exit()

```
print '<header><link rel="stylesheet" type="text/css" href="/jrainey/Tyler_relaxation/
                                                                                                                                                                                                                                                                                                                                                                                                                                 dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              elif form['tau_slow_input_units1'].value=='ps'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   if form['tau_slow_input_units1'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                    ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                    ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                   form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                       print 'Content-Type: text/html/n/n'
                                                                                                                                                                                                                                                                                                                                                             os.environ['PATH']=os.pathsep.join\
                                                                                                                                   import Gnuplot, Gnuplot.funcutils
                                                                                                                                                           from constants_equations import
#!/home/structbi/bin/python2.5
                                                                                       cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      k = (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          K = (10**-9)
                                                                                                                                                                                                                                                                                                                                         main.css" /></header>'
                                                                                                                                                                                import class_storage
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             else:
                                                                                                             math
                                                                  cgi
                                                                                                                                                                                                         0
                                                                                         import
                                                                                                                                                                                                       import
                                            import
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                                                                                                               import
                                                                                                                                                                                                                                                                                                                                                                                                              try:
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```

```
tau_m=float(form['tau_m_extended_input'].value)
                                                                   elif form['tau_slow_input_units2'].value=='ps'
                                                                                                                                                                                                                                                                                                                                                      elif form['tau_fast_input_units'].value=='ps':
if form['tau_slow_input_units2'].value=='ns':
                                                                                                                                                                                                                                                                                 if form['tau_fast_input_units'].value=='ns':
                                                                                                                                                                                                                                               tau_fast=float(form['tau_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               b=float(form['S_squared_slow_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 c=float(form['S_squared_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #print form['tau_fast_input_units'].value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                       tau_fast=tau_fast*(10**-12)
                                                                                                                                                                                                                                                                                                                      tau_fast=tau_fast*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                tau_m = tau_m * (10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 tau_m = tau_m * (10 * * - 9)
                                                                                                    j = (10**-12)
                                 j = (10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       form.keys()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               a=tau_fast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #print
                                                                                                                                             else:
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k=1

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form['tau_slow_low'].value)*k,parameter_max=float(form['tau_slow_high'].value
                                                                                                                             )*j,npar=int(form['npar'].value),field_values=26,model="extended",tau_fast=a,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          input values are number characters</center>'
                                           instance_1=class_storage.tau_slow_varied_plotter(tau_m=w,parameter_min=float(
                                                                                                                                                                                                                         instance_1.plot_1(tau_m=w,parameter_min=float(form['tau_slow_low'].value)*k
                                                                                                                                                                                                                                                                     parameter_max=float(form['tau_slow_high'].value)*j,npar=int(form['npar']
                                                                                                                                                                                                                                                                                                                 value),field_values=26,model="extended",tau_fast=a,S_squared_slow=b,
                                                                                                                                                                             S_squared_slow=b, S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 print '<center>Please ensure all
                                                                                                                                                                                                                                                                                                                                                               S_squared_fast=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    sys.exit()
w = tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 except:
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a dictionary with form info
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    tau_m=float(form['tau_m_input'].value)
                                                                                                                                                                                                                                                                                                                                                                   ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              tau_m=tau_m*(10**-9)
                                                                                                                                                                                                                                       print 'Content-Type: text/html\n\n'
                                                                                                                                                                                                                                                                                                                                                                                                                form=cgi.FieldStorage() #retrieves
                                                                                                                                                                                                                                                                                                                                              os.environ['PATH']=os.pathsep.join\
                                                                                                                                                    from constants_equations import *
                                                                                                                              import Gnuplot, Gnuplot.funcutils
#!/home/structbi/bin/python2.5
                                                                                    cgitb; cgitb.enable()
                                                                                                                                                                       import class_storage
                                                                                                                                                                                                                                                                                                                                                                                                                                                           if form:
                                                                                                         math
                                                               cgi
                                                                                                                                                                                              import os
                                                                                    import
                                          import
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print '<link rel="stylesheet" type="text/css" href="/jrainey/</pre>
                                                                                                                                                                                                elif form['tau_fast_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                         elif form['tau_slow_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                          if form['tau_slow_input_units'].value=='ns':
                                                                                                                        tau_fast=float(form['tau_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                  tau_slow=float(form['tau_slow_input'].value)
                                                                                                                                                if form['tau_fast_input_units'].value=='ns'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         d=float(form['S_squared_fast_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                e=float(form['S_squared_slow_input'].value)
                                                                                                                                                                                                                        tau_fast=tau_fast*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_slow=tau_slow*(10**-12)
                                                                                                                                                                       tau_fast=tau_fast*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                 tau_slow=tau_slow*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Tyler_relaxation/main.css"
tau_m=tau_m*(10**-12)
                                                                                                                                                                                                                                                                          pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    pass
                                                    pass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           c=tau_slow
                                                                                                                                                                                                                                                                                                   b=tau_fast
                                                                           a=tau_m
                          else:
                                                                                                                                                                                                                                                   else:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             else:
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tau_e=1,S_squared=1,model="extended",S_squared_fast=d,S_squared_slow=
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     instance_4=class_storage.R1_single_plotter('R_1','R_1 (s^{-1})',tau_m=a_plotter('R_1','R_1')',R_1 (s^{-1})',tau_m=a_plotter('R_1','R_1')',R_1'',R_1'')', tau_m=a_plotter('R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1','R_1')', tau_m=a_plotter('R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1','R_1
                                                                                                                              tau_e=1, S_squared=1, model="extended", S_squared_fast=d, S_squared_slow
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             print '<center><img src="/relaxation_tmp/%s"></center>' % instance_3.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  =1,S_squared=1,model="extended",S_squared_fast=d,S_squared_slow=e,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         print '<center><img src="/relaxation_tmp/%s"></center>' % instance_1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print '<center><img src="/relaxation_tmp/%s"></center>' % instance_2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         instance_3=class_storage.NOE_single_plotter('NOE','NOE',tau_m=a,tau_
instance_1=class_storage.T1_single_plotter('T_1','T_1 (s)',tau_m=a,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 instance_2=class_storage.T2_single_plotter('T_2','T_2 (s)',tau_m=a,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              output_file.replace('.../Documents','')
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                                                                                                                                                                                                                                           e,tau_fast=b,tau_slow=c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   e,tau_fast=b,tau_slow=c)
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance_2.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          instance_3.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             instance_1.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance_4.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            instance_2.plot()
                                                                                                                                                                                                                                                                                                                                                                          instance_1.plot()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance_3.plot()
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               print '<hr />'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 print '<hr />'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     print
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,tau_e=1,S_squared=1,model="extended",S_squared_fast=d,S_squared_slow
                                                                                                                                                                                                                                                    instance_5=class_storage.R2_single_plotter('R_2','R_2 (s^{-1})',tau_m=a_plotter('R_2','R_2','R_2')',tau_m=a_plotter('R_2','R_2')',R_2'',tau_m=a_plotter('R_2','R_2')',R_2'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R_3'',R
print '<center><img src="/relaxation_tmp/%s"></center>' % instance_4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            print '<center><img src="/relaxation_tmp/%s"></center>' % instance_5
                                                                                    output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        <style type="text/css" media="all">
                                                                                                                                                                                                                                                                                                                                                                                                                                =e,tau_fast=b,tau_slow=c)
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                                                                                                                                                               print '<hr />'
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border-bottom-width: 0;
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<a id="current" href="../../structbi/cgi-bin/single_curve_extended.</pre>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     initial_radio_classical_multi.html">Classical - Multiple Curves</a>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     initial_radio_extended_multi.html">Extended - Multiple Curves</a>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              <a id="other" href="../../structbi/cgi-bin/single_curve.py">
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          <a id="other" href="../../jrainey/Tyler_relaxation/</pre>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        id="a"><a id="other" href="../../jrainey/Tyler_relaxation/</a>
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  id="pageD" class="tabs">
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Classical - Single Curve</a>
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                                                                                                                                                      background: #2E8B57;
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Fast internal correlation time, &tau<sub>fast</sub> --> <input
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                                                                                                                                                                                                                                                                                                                                                                                value="ps"/>
                         <strong> Set the constant parameter values
                                                                                                                                                                                                                                                                                                                                                                                                          name="tau_m_input_units" value="ns"
                                                                                                                                                                                                                                                                                                                                                                                                                                                              s<input type="radio" name="tau_m_input_units" value="s"/>
                                                                                                                                                                                                                                                                                                                            rotational corrrelation time, &tau<sub>m</sub>
                                                                                                                                                                                                                                                                                                                                                    type="text" name="tau_m_input" value="10">
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 type="text" name="tau_fast_input" value="5">
                                                                                                                                                                                                                                                                                                                                                                                type="radio" name="tau_m_input_units"
                                                                                                                                  action="../../structbi/cgi-bin/
                                                                                                                                                          single_curve_extended.py" target="plot_frame">
                                                                                                                                                                                                                                                                        style="border:1px solid">
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                                                                                                                                                                                                                                                                                                                                                                                                          type="radio"
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<input type="text" name="S_squared_fast_input" value="0.85">
                                                                                                                                                                                                                                                                                                                                                                                                                                         <input type="text" name="S_squared_slow_input" value="0.77">
                                                                      value="ps"/>
                      --> <input
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                                                                                               name="tau_slow_input_units" value="ns"
                                                                                                                                              s<input type="radio" name="tau_slow_input_units" value="s"/>
                                                                                                                                                                                                                                                                                              Fast partial order parameter, S<sup>2</sup><sub>fast</sub>
                                                                                                                                                                                                                                                                                                                                                                                                                    Slow partial order parameter, S<sup>2</sup><sub>slow</sub>
                        time, &tau<sub>slow</sub>
                                                                      ps<input type="radio" name="tau_slow_input_units"
                                            type="text" name="tau_slow_input" value="5">
<t
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                                                                                                                                                                                                                                                                     correlation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     submit" value="plot">
                                                                                              ns<input type="radio"
                                                                                                                     checked="yes"/>
                       Slow internal
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sys.exit()

```
form=cgi.FieldStorage() #retrieves a dictionary with form info
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        elif form['tau_m_input_units'].value=='ps':
                                                                                                                                                                                                                                                                                                                                                                                                                                                             if form['tau_m_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                         tau_m=float(form['tau_m_input'].value)
                                                                                                                                                                                                                                                                                                        ([os.environ['PATH'],'/usr/local/bin'])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             tau_m=tau_m*(10**-12)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  tau_m=tau_m*(10**-9)
                                                                                                                                                                                                                     print 'Content-Type: text/html\n\n'
                                                                                                                                                                                                                                                                                 os.environ['PATH']=os.pathsep.join\
                                                                                                                                from constants_equations import *
                                                                                                           import Gnuplot, Gnuplot.funcutils
#!/home/structbi/bin/python2.5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         pass
                                                                cgitb; cgitb.enable()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               a=tau_m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    else:
                                                                                                                                                     import class_storage
                                                                                                                                                                                                                                                                                                                                                                                              if form:
                                                                                     import math
                                            cgi
                                                                import
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```
print '<center><img src="/relaxation_tmp/%s"></center>' % instance_1.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               print '<center><img src="/relaxation_tmp/%s"></center>' % instance_2.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         instance_1=class_storage.T1_single_plotter('T_1','T_1 (s)',a,b,c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             instance_2=class_storage.T2_single_plotter('T_2','T_2 (s)',a,b,c)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               instance_3=class_storage.NOE_single_plotter('NOE','NOE',a,b,c)
                                                                                                                                                                                                                                                                                                                                                                                                                              print '<link rel="stylesheet" type="text/css" href="/jrainey/</pre>
                                                                                                              elif form['tau_e_input_units'].value=='ps':
                                 if form['tau_e_input_units'].value=='ns':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                                                                c=float(form['S_squared_input'].value)
tau_e=float(form['tau_e_input'].value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Tyler_relaxation/main.css" />'
                                                                                                                                                     tau_e=tau_e*(10**-12)
                                                                        tau_e=tau_e*(10**-9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance_1.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance_2.csv_printer()
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                instance_1.plot()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 instance_2.plot()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            print '<hr />'
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instance_4=class_storage.R1_single_plotter('R_1','R_1 (s^{-1})',a,b,c)
                                                                                                                                                                                                          instance_5=class_storage.R2_single_plotter('R_2','R_2 (s^{-1})',a,b,c)
print '<center><img src="/relaxation_tmp/%s"></center>' % instance_3.
                                                                                                                                                                                                                                                                               % instance_5
                                                                                                                                       src="/relaxation_tmp/%s"></center>' % instance_4
                                                                                                                                                                                                                                                                               print '<center><img src="/relaxation_tmp/%s"></center>'
                       output_file.replace('../Documents','')
                                                                                                                                                             output_file.replace('../Documents','')
                                                                                                                                                                                                                                                                                                     output_file.replace('../Documents','')
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                                                                                                                 instance_4.csv_printer()
                                                                                                                                                                                                                                                       instance_5.csv_printer()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       <style type="text/css"</pre>
                                                                                                                                         print '<center><img
                                                                                           instance_4.plot()
                                                                                                                                                                                                                                 instance_5.plot()
                                                                                                                                                                                     print '<hr />'
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initial_radio_classical_multi.html">Classical - Multiple Curves</a>
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id="other" href="../../structbi/cgi-bin/single_curve_extended.py
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       s<input type="radio" name="tau_m_input_units" value="s"/>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        value="ps"
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                                                                                                                                                                                                                                                                                                                                                           rotational corrrelation time, &tau<sub>m</sub>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Effective internal correlation time, &tau<sub>e</sub>
                                                                                                                                                                                                                                                                                                                                                                                      type="text" name="tau_m_input" value="10">
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          input type="text" name="tau_e_input" value="50">
                                                                                                                      <strong> Set the constant parameter values </strong><br />
                                                                                                                                                                                                                                                                                                                                                                                                                    ps<input type="radio" name="tau_m_input_units"
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                               Single Curve</a>
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                                                                                                                                                                             target="plot_frame">
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id="d"><a
                               ">Extended
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<input style="position:absolute;top:170px;right:600px" type="</pre>
                                                                                                                                                                                                                                                                             print '<center>Please ensure all input values are number characters</center>'
                  <input type="text" name="</pre>
                  Order parameter, S<sup>2</sup> -->
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                                    S_squared_input" value="0.85">

                                                                                                                                     submit" value="plot">
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Appendix D: Source Code For FGFR3 MD Simulation Analysis

D.1 Introduction

This appendix contains the Python (MDAnalysis-based) source code used for parsing the production-length GROMACS trajectories for the FGFR3 CG-MD replicate simulations.

D.2 Python Source Code For Trajectory Parsing With MDAnalysis

Listing D.15: This module (analyze-FGFR3-dimer-simulations.py) recursively moves

```
directory with symlinks to the original production-length
                                                                                                                                                                                                                                                                                                 over
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      heterodimer_replicate_3','heterodimer_replicate_4','heterodimer_replicate_5','heterodimer_replicate_6','heterodimer_replicate_8','
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = ['wildtype_dimer_replicate_1','wildtype_dimer_replicate_2','
                                                                                                                                                                                                                                                                                         #folders for primary and secondary dimer interface testing (split trajectory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   '''folder_name_list = ['heterodimer_replicate_1','heterodimer_replicate_2','
                                                                                                                                                                                                                                                                                                                                                             = ['primary_interface_mut4','secondary_interface_mut4']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               \#folder\_name\_list = ['GpA\_dimer\_replicate\_1', 'GpA\_dimer\_replicate\_2']
                                                                                                                                                                               dimer_symlink_directory = '/sansom/sc2/bioc1009/Documents/FGFR3_work/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'wildtype_dimer_replicate_10']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'wildtype_dimer_replicate_4',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'wildtype_dimer_replicate_6';
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'wildtype_dimer_replicate_8',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    GpA_dimer_replicate_3', 'GpA_dimer_replicate_4']
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         ['mutant_dimer_replicate_4']
                                                                                                                                                                                                                                                                                                                            transition in mutant homodimer replicate 4):
                                                                                                               0
                                                                                                          dimer_geometric_tools,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #FGFR3 heterodimer and mutant folders:
                                                                                                                                                                                                                  dimer_batch_analysis_symlink/'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           wildtype_dimer_replicate_9',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    wildtype_dimer_replicate_3',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    wildtype_dimer_replicate_7',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #GpA control folders (first 4):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   wildtype_dimer_replicate_5',
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in
files organized
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                  folders.'''
                                                                                                          import MDAnalysis,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #FGFR3 WT folders:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #folder_name_list
                                                                                                                                                                                                                                                                                                                                                                 #folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                                            set:
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heterodimer_replicate_9','heterodimer_replicate_10','mutant_dimer_replicate_1', 'mutant_dimer_replicate_2','mutant_dimer_replicate_3','mutant_dimer_replicate_4',' mutant_dimer_replicate_5','mutant_dimer_replicate_6','mutant_dimer_replicate_7', "mutant_dimer_replicate_8','mutant_dimer_replicate_9','mutant_dimer_replicate_10']'''	#all FGFR3 folders: folder_name_list = ['heterodimer_replicate_1','heterodimer_replicate_2',' heterodimer_replicate_3','heterodimer_replicate_4','heterodimer_replicate_5','	', 'heterodimer_replicate_7'', 'heterodimer_replicate_1'', 'heterodimer_replicate_1'', 'heterodimer_replicate_1'', 'heterodimer_replicate_1'', 'mutant_dimer_replicate_3'', 'mutant_dimer_replicate_5'', 'mutant_dimer_replicate_6'', 'mutant_dimer_replicate_6'', 'mutant_dimer_replicate_6'', 'mutant_dimer_replicate_6'', 'mutant_dimer_replicate_6'', 'wildtype_dimer_replicate_6'', 'wildtype_dimer_replicate_6'', 'wildtype_dimer_replicate_6''', 'wildtype_dimer_replicate_6'''	wildtype_dimer_replicate_4','wildtype_dimer_replicate_5','wildtype_dimer_replicate_6', 'wildtype_dimer_replicate_7','wildtype_dimer_replicate_8','wildtype_dimer_replicate_9' 'wildtype_dimer_replicate_10']	#all folders without lipid flip-flop (FGFR3 WT dimer replicates 1 and 4 removed): "" folder_name_list = ['heterodimer_replicate_1','heterodimer_replicate_2',' heterodimer_replicate_3','heterodimer_replicate_4','heterodimer_replicate_5',' heterodimer_replicate_6','heterodimer_replicate_7','heterodimer_replicate_8','	heterodimer_replicate_9','heterodimer_replicate_10','mutant_dimer_replicate_1', 'mutant_dimer_replicate_2','mutant_dimer_replicate_3','mutant_dimer_replicate_4',' mutant_dimer_replicate_5','mutant_dimer_replicate_6','mutant_dimer_replicate_7', "mutant_dimer_replicate_8','mutant_dimer_replicate_9','mutant_dimer_replicate_10','
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```
'alpha_carbon_popc_and_centered_trajectory.xtc') #no_jump_trajectory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    list_of_xtc_file_paths.append(dimer_symlink_directory + folder + '/' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    for pdb_file, xtc_file, folder_name in list_data_paths(dimer_symlink_directory
'wildtype_dimer_replicate_5','wildtype_dimer_replicate_6','wildtype_dimer_replicate_7
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   list_of_pdb_file_paths.append(dimer_symlink_directory + folder + '/'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      = zip(list_of_pdb_file_paths,list_of_xtc_file_paths,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'alpha_carbon_popc_final_snapshot.gro') #,centered_CA_POPC.pdb;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             differently
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         files should be accepted now too; 'original_final_snapshot.gro'
                                                                                                                                                                                                                                                                                                                2) in the
                                                                                                                                                                                                                                                              a list of tuples with pairs of pdb (index 0) and xtc (index 1)
                                                                                                                                GpA_dimer_replicate_3', 'GpA_dimer_replicate_4', 'GpA_dimer_replicate_5']'''
                                                                                   'wildtype_dimer_replicate_10', 'GpA_dimer_replicate_1', 'GpA_dimer_replicate_2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   selected)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      create_universe_selections(dimer_symlink_directory,folder_name_list)
                                                                                                                                                                                                                                                                                                         a given simulation folder. The name of the folder is (index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for folder in folder_name_list: #files names can be changed for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        processed trajectories (i.e., simplified and centered or not)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        system
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              xtc ,'original_trajectory.xtc', , 'interface.xtc'
                                                                                                                                                                                                                        list_data_paths(dimer_symlink_directory,folder_name_list):
                                          "wildtype_dimer_replicate_8", "wildtype_dimer_replicate_9"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   atoms in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 (all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            a nested list of [universe
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       MDAnalysis objects, folder_name]'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              return combined_path_pair_list
                                                                                                                                                                                                                                                                                                                                                                                                     list_of_pdb_file_paths=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                list_of_xtc_file_paths=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         combined_path_pair_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   folder_name_list)
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```
GpA_local_thickness_pre_dimer_list, GpA_local_thickness_post_dimer_list, skip_frames
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 WT_FGFR3_local_thickness_pre_dimer_list, WT_FGFR3_local_thickness_post_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          hetero_FGFR3_local_thickness_post_dimer_list, mutant_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       hetero_FGFR3_distal_thickness_list, hetero_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                 plot:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       dimer_symlink_directory,folder_name_list, heterodimer_helix_2_x_coord_list
                                                                                                                                                   overall
universe_list.append([MDAnalysis.Universe(pdb_file,xtc_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                mutant_FGFR3_local_thickness_post_dimer_list, GpA_distal_thickness_list,
                                                                                                                                                   data for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      def main(primary_count_list, secondary_count_list, other_count_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                wildtype_helix_2_y_coord_list, WT_FGFR3_distal_thickness_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                         plot' data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list,
                                                                                                                                                 FGFR3 'top five closest' contact
                                                                                                                                                                                                                                                                                                                                                                                                         FGFR3 'thermal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               mutant_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                         #initialize lists for dumping overall
                                                                                                                                                                                                                                                                                                                                                                                                                                               II
                                                                                                                                                                                                                                                                                                                                                                                                                                            heterodimer_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               heterodimer_helix_2_y_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  folder_name])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    mutant_helix_2_x_coord_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     mutant_helix_2_y_coord_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         wildtype_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              wildtype_helix_2_y_coord_list
                                                                         universe_list
                                                                                                                                                                                                                       for dumping overall
                                                                                                                                                                                                                                                                                                                                 secondary_count_list=[]
                                                                                                                                                                                    #mutant_overall_list =
                                                                                                                                                                                                                       #wildtype_overall_list
                                                                                                                                                                                                                                                                                              primary\_count\_list=[]
                                                                                                                                                                                                                                                          #hetero_overall_list
                                                                                                                                                                                                                                                                                                                                                                      other_count_list=[]
                                                                                return
                                                                                                                                                   #lists
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```
closest_approach_outfile, GpA_top_five_outfile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     universe_object, skip_frames, output_file = closest_approach_outfile)
                                                                                                                                                                                                functions '''
                                                                          relative_Z_outfile,local_thickness_outfile,frame_position_outfil
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_object, skip_frames, output_file=helix_tilt_output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         universe_object, skip_frames, output_file=helix_tilt_output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_object, skip_frames, output_file=FGFR3_top_five_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    _geometric_tools.GpA_helix_tilt_vs_bilayer_normal(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          geometric_tools.top_five_closest_residues_FGFR3(folder_name,
                                        FGFR3_top_five_outfile, relative_helical_motion_outfile, fixed_thermal_outfile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #dimer_geometric_tools.geo_Z_tracking(folder_name, universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    universe_object, skip_frames, output_file=GpA_top_five_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #dimer_geometric_tools.top_five_closest_residues_GpA(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #dimer_geometric_tools.helix_tilt_vs_bilayer_normal(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   geoZ_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                            t o
                                                                                                                                                                                                                                                                                                                                                                                                   gnuplot
                                                                                                                                                                                                                                                                                                                     s.chdir(dimer_symlink_directory + folder_name) #move to the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #dimer_geometric_tools.merged_top_five_FGFR3(mutant_overall_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #dimer_geometric_tools.relative_helical_motion(folder_name
                                                                                                                                                                                                  various
                                                                                                                                                                                                                                                                                                                                                                                                                                            them
                                                                                                                                                                                                                                                                                                                                                               executing data processing
                                                                                                                  lipid_shell_outfile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 overall_list, folder_name
                                                                                                                                                                                                                                         create_universe_selections(
                                                                                                                                                                                                                                                                                                                                                                                                     be in
                                                                                                                                                                                                                                                                                                                                                                                                                                            you are not needing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       skip_frames, system_name='FGFR3', output_file =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               .geometric_tools.closest_approach_GpA(folder.
                                                                                                                                                                                            calling
                                                                                                                                                                                                                                                                                                                                                                                                 should
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   output_file=
                                                                                                                                                                                                ру
                                                                                                                                                                                                                                                                                                                                                                                                   output
                                                                                                                                                                                                symlink directories
                                                                                                                      bilayer_tracking_file,
                                                                                                                                                                                                                                                                               dimer_symlink_directory, folder_name_list):
                                                                                                                                                                                                                                                                                                                                                                                                 functions;
                                                                                                                                                                                                                                                                                                                                                                                                                                          comment out functions when
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               wildtype_overall_list,hetero_
                                                                                                                                                                                                                                                                                                                                                             appropriate directory before
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     universe_object, skip_frames)
                                                                                                                                                                                                                                       for universe_object, folder_name in
geoZ_outfile,
                                                                                                                                                                                                                                                                                                                                                                                                   processing
                                                                                                                                                                                                '''prints data to files in
                                                                                                                      efficient_contacts_outfile,
helix_tilt_output_file,
                                                                                                                                                                                                                                                                                                                                                                                                     data
                                                                                                                                                                                                                                                                                                                                                                                                                                            #just
                                                                                                                                                            flip_flop_outfile)
                                                                              bilayer_outfile,
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343

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#dimer_geometric_tools.fixed_helix_thermal(folder_name, universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #dimer_geometric_tools.geo_Z_tracking_relative_to_bilayer(folder_name,
                                                                                                                                                                                                                                                              , skip_frames, dimer_symlink_directory, create_universe_selections,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #dimer_geometric_tools.frame_abstracted_relative_position(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     , output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'FGFR3', output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          universe_object, skip_frames, system_name='FGFR3', output_file=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          universe_object, skip_frames, dimer_symlink_directory, outfile=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         create_universe_selections, heterodimer_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #dimer_geometric_tools.closest_contacts_efficient(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             system_name='GpA', output_file=
                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.fixed_helix_thermal_merged(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #dimer_geometric_tools.local_bilayer_thickness(folder_name,
                                                    #dimer_geometric_tools.relative_helical_motion(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #dimer_geometric_tools.track_bilayer_thickness(folder_name,
                                                                                                                                                                                                                                                                                                                system_name='FGFR3', output_file=fixed_thermal_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                         universe_object, skip_frames, dimer_symlink_directory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              universe_object, skip_frames, dimer_symlink_directory
                                                                                                                                                          relative_helical_motion_outfile, system_name='FGFR3')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #dimer_geometric_tools.distance_versus_crossing_angle()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     = 'FGFR3',
  system_name='GpA')
                                                                                                         universe_object, skip_frames, output_file=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   universe_object, skip_frames, system_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           system_name
relative_helical_motion_outfile,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              wildtype_helix_2_y_coord_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             create_universe_selections,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          efficient_contacts_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          local_thickness_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    frame_position_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               relative_Z_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         bilayer_outfile)
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#dimer_geometric_tools.box_size_assessment(folder_name, universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.flip_flop_tracker(folder_name, universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    skip_frames, outfile_name = bilayer_tracking_file, local_definition
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #dimer_geometric_tools.optimize_leaflet_selection_cutoff(folder_name
                                                                                                       #dimer_geometric_tools.closest_approach_representative(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           = lipid_shell_outfile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       GpA_distal_thickness_list, GpA_local_thickness_pre_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    15.5)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #dimer_geometric_tools.count_lipids_in_local_shell(folder_name
                                                                                                                                                                                                                                                                #dimer_geometric_tools.absolute_delta_Z_and_closest_approach()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             = 15.5, system_name = 'GpA')
                                                    #dimer_geometric_tools.correlate_helixcrossing_polar_theta()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #dimer_geometric_tools.bilayer_thickness_average_results(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       skip_frames, outfile_name = flip_flop_outfile, cutoff
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #dimer_geometric_tools.analyze_leaflets(folder_name,
                                                                                                                                                           universe_object, skip_frames, system_name='FGFR3')
                                                                                                                                                                                                              #dimer_geometric_tools.absolute_value_Z_tracking()
#dimer_geometric_tools.cartesian_to_polar_theta()
                                                                                                                                                                                                                                                                                                               #dimer_geometric_tools.split_Z_file(folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  mutant_FGFR3_local_thickness_post_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    hetero_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         hetero_FGFR3_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                mutant_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_object, skip_frames, outfile_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              WT_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  WT_FGFR3_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       = 15.5, system_name = 'GpA')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  hetero_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              mutant_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           WT_FGFR3_distal_thickness_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                local_definition = 16, cutoff
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_object)
                                                                                                                                                                                                                                                                                                                                                                                                                              , skip_frames)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       cutoff
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dimer_symlink_directory,folder_name_list, heterodimer_helix_2_x_coord_list,
                                                                                #dimer_geometric_tools.simple_moving_average_polar_theta(folder_name,
                                                                                                                                                                                                                                                                                                                                                    global lists for the overall bilayer thickness averaging
                                                                                                                                                        dimer_geometric_tools.interface_filtered_merged_top_five_FGFR3
                                                                                                                                                                                               primary_count_list, secondary_count_list, other_count_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         main(primary_count_list, secondary_count_list, other_count_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              wildtype_helix_2_y_coord_list,WT_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list,
GpA_local_thickness_post_dimer_list,folder_name
                                                                                                                                                                                                                                    folder_name, universe_object, skip_frames)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          □
||
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          □
=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            hetero_FGFR3_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             mutant_FGFR3_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     hetero_FGFR3_local_thickness_pre_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      mutant_FGFR3_local_thickness_pre_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   WT_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          WT_FGFR3_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     WT_FGFR3_local_thickness_pre_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               □
||
                                         6.0)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               mutant_FGFR3_distal_thickness_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          GpA_local_thickness_pre_dimer_list =
                                            II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               GpA_local_thickness_post_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                hetero_FGFR3_distal_thickness_list
                                         dimerization_criterion
                                                                                                                                                                                                                                                                                                                                                                                                                              WT_FGFR3_distal_thickness_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  GpA_distal_thickness_list =
                                                                                                                   window_size = 10
                                                                                                                                                                                                                                                                                                               __name__ == "__main__":
                                                                                                                                                                                                                                                                                                                                                      #initialize some
                                                                                                                                                                                                                                                                                                                                                                                             function:
                                                                                                                                                                                                                                                                                                                   ij
                                                                                                                                                                                                                                                                                                                        111
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    115
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                                                                                                                                                                  109
                                                                                                                                                                                                                                                                                                                                                                                                                                        113
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 123
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              125
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       121
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local_thickness_outfile='local_bilayer_thickness.out', frame_position_outfile lipid_shell_outfile = 'lipid_shell_count.out', flip_flop_outfile = 'flip_flop closest_contacts_full.out', bilayer_tracking_file = 'bilayer_thickness.out', WT_FGFR3_local_thickness_post_dimer_list,hetero_FGFR3_distal_thickness_list, , geoZ_outfile= GpA_distal_thickness_list, GpA_local_thickness_pre_dimer_list, GpA_local_thickness_post_dimer_list, testing_MDA_contacts.out', GpA_top_five_outfile='top_five_testing.out' 'relative_Z.out' ='frame_correlated_position_noskip.out', efficient_contacts_outfile= relative_helical_motion_outfile='relative_helical_nojump.out' skip_frames=10, helix_tilt_output_file='dimer_tilt_test.out' fixed_thermal_outfile='fixed_thermal.out', bilayer_outfile=' DualZ_tracking_nocenter.out', closest_approach_outfile = bilayer_thickness_tracking.out', relative_Z_outfile = FGFR3_top_five_outfile='interface_top_five.out', mutant_FGFR3_local_thickness_post_dimer_list, hetero_FGFR3_local_thickness_post_dimer_list, hetero_FGFR3_local_thickness_pre_dimer_list, mutant_FGFR3_local_thickness_pre_dimer_list, mutant_FGFR3_distal_thickness_list,

WT_FGFR3_local_thickness_post_dimer_list,hetero_FGFR3_distal_thickness_list GpA_distal_thickness_list, GpA_local_thickness_pre_dimer_list, GpA_local_thickness_post_dimer_list) mutant_FGFR3_local_thickness_post_dimer_list, hetero_FGFR3_local_thickness_post_dimer_list, mutant_FGFR3_local_thickness_pre_dimer_list, hetero_FGFR3_local_thickness_pre_dimer_list, WT_FGFR3_local_thickness_pre_dimer_list, mutant_FGFR3_distal_thickness_list,

#dimer_geometric_tools.bilayer_stats(WT_FGFR3_distal_thickness_list,

#print bilayer thickness results to a file:

```
heterodimer_helix_2_y_coord_list, output_file='/sansom/sc2/bioc1009/Documents
                                                                                                                                                                                                                                                                                                 mutant_helix_2_x_coord_list, helix_2_y_coord_list=mutant_helix_2_y_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  wildtype_helix_2_y_coord_list , output_file='/sansom/sc2/bioc1009/Documents/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.parse_overall_FGFR3_top_five_data(mutant_overall_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   dimer_geometric_tools.interface_filtered_parse_overall_FGFR3_top_five_data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        analysis
                                                                                                                                                 /FGFR3_work/dimer_batch_analysis_symlink/overall_thermal_plot_data,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  FGFR3_work/dimer_batch_analysis_symlink/overall_thermal_plot_data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        primary_count_list, secondary_count_list, other_count_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        qo
                                                                                                                                                                                                                                                                                                                                              , output_file='/sansom/sc2/bioc1009/Documents/FGFR3_work,
                                                  heterodimer_helix_2_x_coord_list, helix_2_y_coord_list=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           to
#dimer_geometric_tools.thermal_bins(helix_2_x_coord_list=
                                                                                                                                                                                                                                                 #dimer_geometric_tools.thermal_bins(helix_2_x_coord_list=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.thermal_bins(helix_2_x_coord_list=
                                                                                                                                                                                                                                                                                                                                                                                                dimer_batch_analysis_symlink/overall_thermal_plot_data,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    wildtype_helix_2_x_coord_list, helix_2_y_coord_list=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        need
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        contacts'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       wildtype_overall_list,hetero_overall_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      FGFR3 'top five
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 wildtype_dimer_thermal_merged.out')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   outside of the trajectory looping:
                                                                                                                                                                                                                                                                                                                                                                                                                                                     mutant_dimer_thermal_merged.out')
                                                                                                                                                                                               heterodimer_thermal_merged.out')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #for overall parsing of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    139
            133
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 135
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  137
```

data spread over many directories produced by the high-throughput SIDEKICK sim-This module (analyze_sidekick_FGFR3_dimer_simulations.py) serves ulation program (created by Benjamin Hall, University of Oxford)

```
FGFR3,
                                                                        at: /sbcb/sn2,
simulations in DPPC (only lipid option
                                                                                                                                                                                                                                             simulations complete for each FGFR3 condition.'''
                                                                                                                                                                                                                                                                                                                                                                                                                                         sequences for the above three mentioned FGFR3
                                                                                                                                                                                                                                                                                                                                            of high throughput results: wild-type
                                                                                                                                                                                                                                                                                                                                                                                         sidekick_data_directory = '/sbcb/sn2/hall/nfsmount/Data/FGFR3_dimers_Tyler/Bond/
                                                                                                                                               different
                                                                                                                                                                     a template (this
                                                                                                                                                                                            analysis module for non-SIDEKICK results)
                                                                        located
                                                                                                                                               to deal with this
                                                                     copy locally) is
                                                                                                                                                                     but I am using the previous stuff as
                                                                                                                                                                                                                                                                                                                                                                    simulation data:
                                                                                                                                               current local parsing script
                                                                        The high-throughput data (far too much space to
  dimer
                                                                                               all/nfsmount/Data/FGFR3_dimers_Tyler/Bond
                                                                                                                                                                                                                                                                                             dimer_geometric_tools, os
                                                                                                                                                                                                                                                                                                                                           three subdivisons
  for
                                                                                                                                                                                                                                                                                                                                                                    mutant homodimer
  '''Analyzing the SIDEKICK FGFR3 data
                          the moment)
                                                                                                                                                                                                                                                                                                                                                                                                                                            the
                                                                                                                                                                                              local
                                                                                                                                                                                                                                                                                                                                                                                                                                            #the subdirectory names reflect
                                                                                                                                                                                                                                             500 ns
                                                                                                                                                                                               the
                        SIDEKICK dimers at
                                                                                                                                                                                                                                               +06
                                                                                                                                                                                                                                                                                                                                             directory has
                                                                                                                                                                                              copy of
                                                                                                                                                                        directory structure,
                                                                                                                                                adjust my
                                                                                                                                                                                                                                                 are
                                                                                                                                                                                                                                                                                                                                                                      and
                                                                                                                                                                                                                                                                                            import MDAnalysis,
                                                                                                                                                                                                                                               like there
                                                                                                                                                                                                                                                                                                                                                                   heterodimer,
                                                                                                                                                                                                ಡ
                                                                                                                                                                                                ล
                                                                                                                                               Will have to
                                                                                                                                                                                                                                                                                                                                             #this data
                                                                                                                                                                                                                                               Looks
                                                                                                                                                                                                                                                                                                                                                                                                                                                14
                                                                                                                                                                                                                                                                                                                                                                                                 12
                                                                                                                                                                                                                                                                                                                           10
```

RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR_____--RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR

folder_name_list = ['RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR

RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR_____/DPPC/1/',

DPPC/1/', 'RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR_

RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR

```
use other
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_list.append([MDAnalysis.Universe(gro_file,xtc_file), full_path
  the
                                                                                                                                                                                                                                                                                                                                                                                                                                                             for gro_file, xtc_file, full_path in combined_path_pair_list:#slice this for
                                                                                                                                                                                                                                            combined_path_pair_list = zip(list_of_gro_file_paths,list_of_xtc_file_paths
                                                                                                                                     + 'em.gro') #the starting
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         plot:
                                                                                                                                                                                     list_of_xtc_file_paths.append(path + '/' + 'center.xtc') #can
                                                                                                                                                                                                                                                                                                                                                                                 selected)
 (index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            overall
                                                                                                                                                                                                                  options if needed: t_0.xtc is the unfiltered trajectory
path of the folder is
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            for
                                                                                                                                                                                                                                                                                                                                                                                 system
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            plot' data:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      testing small portions of data set (less replicates)
                                                                                                                                                                                                                                                                                                                                                                                   ಡ
                                                                                                                                                                                                                                                                                                                                                                                 in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            contact
                                                                                                                                   list_of_gro_file_paths.append(path + '/'
                                                                                                                                                                                                                                                                                                                                                                               '''Creates a nested list of [universe (all atoms
                                                                                                                                                                                                                                                                                                                                                   create_universe_selections(combined_path_pair_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            FGFR3 'thermal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            closest'
                                                                                                                                                                                                                                                                                                                                                                                                      MDAnalysis objects, full_path string]'''
 simulation folder. The full
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          FGFR3 'top five
                                                                                                        path in list_of_full_paths:
                                                                                                                                                                                                                                                                                               combined_path_pair_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #initialize lists for dumping overall
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      heterodimer_helix_2_x_coord_list =
                                                    list_of_gro_file_paths=[]
                                                                                list_of_xtc_file_paths=[]
                                                                                                                                                                                                                                                                     list_of_full_paths)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       universe_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          dumping overall
                                                                                                                                                                                                                                                                                                                                                                                                                                universe_list=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #wildtype_overall_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #hetero_overall_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #mutant_overall_list
  for a given
                                                                                                                                                                                                                                                                                                   return
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            for
                                                                                                                                                                                                                                                                                                                                                       def
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              26
                                                                                                                                                                                                                                                                                                                                                                                 <sup>4</sup>
351
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```

```
universe_object, skip_frames, output_file = closest_approach_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               executing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     be re-run:
                                                                                                                                                                                                                                                                                                                                                                                                directories
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        gnuplot format:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  universe_object, skip_frames, output_file=helix_tilt_output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               universe_object, skip_frames, output_file=helix_tilt_output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 universe_object, skip_frames, output_file=FGFR3_top_five_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #dimer_geometric_tools.GpA_helix_tilt_vs_bilayer_normal(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #dimer_geometric_tools.geo_Z_tracking(folder_name, universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #dimer_geometric_tools.top_five_closest_residues_FGFR3(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      universe_object, skip_frames, output_file=GpA_top_five_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #dimer_geometric_tools.helix_tilt_vs_bilayer_normal(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #dimer_geometric_tools.top_five_closest_residues_GpA(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               os.chdir(path_name) #move to the appropriate directory before
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     them to
                                                                                                                                                                                                                                                                                                                                                                                                  other
                                                                                                                                                                                                                                                                                                                                                     wildtype_helix_2_y_coord_list,skip_frames,efficient_contacts_outfile):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #dimer_geometric_tools.closest_approach_GpA(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            output should be in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 you are not needing
                                                                                                                                                                                                                                                                                                                                                                                                    OL
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            skip_frames, system_name='FGFR3', output_file =
                                                                                                                                                                                                                                                                          heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                analyzed data to files in replicate subfolders
                                                                                                                                                                                                                                                                                                                 mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list
                                                                                                                                                                                                                                      def main(universe_list,heterodimer_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           for universe_object,path_name in universe_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            functions;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   comment out functions when
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        functions
                                                                                                                                                                                                                                                                                                                                                                                                                                  by calling various functions'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #call data processing
data processing
   heterodimer_helix_2_y_coord_list
                                         wildtype_helix_2_y_coord_list
                                                                                                                    wildtype_helix_2_x_coord_list
                                                                            mutant_helix_2y_coord_list
                                         mutant_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #just
                                                                                                                                                                                                                                                                                                                                                                                              '''Prints
                                                   28
                                                                                                                              09
                                                                                                                                                                                                                                                                                                                                                                                                        64
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                                                                                                                                                                                                           62
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        20
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ^{72}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          352
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#dimer_geometric_tools.fixed_helix_thermal(folder_name, universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #dimer_geometric_tools.geo_Z_tracking_relative_to_bilayer(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   create_universe_selections,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #dimer_geometric_tools.frame_abstracted_relative_position(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   = 'FGFR3', output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              universe_object, skip_frames, system_name='FGFR3', output_file=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          skip_frames, sidekick_data_directory, outfile=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
#dimer_geometric_tools.merged_top_five_FGFR3(mutant_overall_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      create_universe_selections, heterodimer_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.fixed_helix_thermal_merged(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           , output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #dimer_geometric_tools.local_bilayer_thickness(folder_name,
                                                                                                                                                                                                                                                                                                         #dimer_geometric_tools.relative_helical_motion(folder_name,
                                                                                                                                                    #dimer_geometric_tools.relative_helical_motion(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #dimer_geometric_tools.track_bilayer_thickness(folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    thermal_outfile)
                                                   .list,hetero_overall_list, folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     universe_object, skip_frames, sidekick_data_directory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        .data_directory
                                                                                                                                                                                                                                                                                                                                                                                                             relative_helical_motion_outfile, system_name='FGFR3']
                                                                                                                                                                                                                                                        relative_helical_motion_outfile, system_name='GpA')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           create_universe_selections, system_name='GpA'
                                                                                                                                                                                                                                                                                                                                                            universe_object, skip_frames, output_file=
                                                                                                                                                                                                          universe_object, skip_frames, output_file=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     universe_object, skip_frames, system_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              sidekick_data_directory,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            system_name='FGFR3', output_file=fixed_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        universe_object, skip_frames, sidekick_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        wildtype_helix_2_y_coord_list)
                                                                                                         universe_object, skip_frames)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         local_thickness_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  relative_Z_outfile)
                                                     wildtype_overall_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               bilayer_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 , skip_frames,
```

```
#dimer_geometric_tools.box_size_assessment(folder_name, universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #dimer_geometric_tools.closest_contacts_efficient_SIDEKICK(path_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #dimer_geometric_tools.fixed_helix_thermal_merged_SIDEKICK(path_name,
                                                                                                                                                                                            #dimer_geometric_tools.closest_approach_representative(folder_name,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      wildtype_helix_2_y_coord_list, skip_frames=10,efficient_contacts_outfile=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             system_name = 'FGFR3', output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      universe_object, skip_frames, heterodimer_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                = full_paths(sidekick_data_directory, folder_name_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                      _approach()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list
                                                                                                                                            #dimer_geometric_tools.correlate_helixcrossing_polar_theta()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            universe_list = create_universe_selections(combined_path_pair_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
                                                #dimer_geometric_tools.distance_versus_crossing_angle()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        mutant_helix_2_y_coord_list, wildtype_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         combined_path_pair_list = list_data_paths(list_of_full_paths)
                                                                                                                                                                                                                                        universe_object, skip_frames, system_name='FGFR3')
                                                                                                                                                                                                                                                                                                                                    #dimer_geometric_tools.absolute_delta_Z_and_closest
                                                                                                                                                                                                                                                                                      #dimer_geometric_tools.absolute_value_Z_tracking()
                                                                                           #dimer_geometric_tools.cartesian_to_polar_theta()
                                                                                                                                                                                                                                                                                                                                                                                  #dimer_geometric_tools.split_Z_file(folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            main(universe_list,heterodimer_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     wildtype_helix_2_y_coord_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             efficient_contacts_outfile)
frame_position_outfile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                , skip_frames)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             list_of_full_paths
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               __main___
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           --name-
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           86
                                                                                                          86
                                                                                                                                                                                                                                                                                                                                                  90
                                                                                                                                                                                                                                                                                                                                                                                                                                              92
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           94
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               96
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          100
                                                                                                                                                                                                      88
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      102
```

the οĘ each in position 2 relative helix binning function for conditions three FGFR3

wildtype_helix_2_y_coord_list, outfile_path='/sansom/sc2/bioc1009/Documents/ #dimer_geometric_tools.thermal_bins_SIDEKICK(wildtype_helix_2_x_coord_list, FGFR3_work/sidekick_dimer_batch_analysis/thermal_interface_results/ WT_thermal_fixed.out')

Documents/FGFR3_work/sidekick_dimer_batch_analysis/thermal_interface_results/ #dimer_geometric_tools.thermal_bins_SIDEKICK(heterodimer_helix_2_x_coord_list, heterodimer_helix_2_y_coord_list, outfile_path='/sansom/sc2/bioc1009 hetero_thermal_fixed.out')

mutant_helix_2_y_coord_list , outfile_path='/sansom/sc2/bioc1009/Documents, #dimer_geometric_tools.thermal_bins_SIDEKICK(mutant_helix_2_x_coord_list, FGFR3_work/sidekick_dimer_batch_analysis/thermal_interface_results. mutant_thermal_fixed.out')

isting D.17: This module (dimer_geometric_tools.py) serves as a library of functions parse the FGFR3 dimer replicate MD trajectories

```
output_file)
                                                                                                                                                                                                                                                                    FGFR3
                                                                                                                                                                                                                                                                                                        This function should
                                                                                                                             ಡ
                                                                                                                           in
                                                                                                                         particles
trajectories.'''
                                                                                                                                                                                                                                                                                                                                           reduce the amplitude
                                                                                                                                                                                                                                                                    GpA
                                                                                                                                                                                                                                                                                                                                                                                                               #select
                                                                                                                                                                                                                                                                     the
                                                                                                                                                                                                                                    system_name,
                                                                                                                                                                                                                                                                                                                                                           (i.e., since
                                                                                                                          CA
                                                                                                                                                                                                                                                                     οĮ
                                                                                                                           for
                                                                                                                                                                                                                                                                   geometric center for each
                                                                                                                                                                                                                                                                                                       frame interval.
                                                                                                                                                                                                                                                                                      specified
DIMER
                                                                                                                            mass
                                                                                                                                                                                                                                 geo_Z_tracking(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                               resid
                                                                                                                           of
                                                                                                                                                                                                                                                                                                                                          centered by GROMACS trjconv because centering will
                                                                                                                                                                                                                                                                                                                                                            1 relative to helix
                                                                                                                                                                                                                                                                                                                         only be used on trajectories that have not been
information from FGFR3
                                                                                                                            center
                                                                                                                                                                                                                                                                                        ಡ
                                                                                                                                                                                                                                                                                                                                                                                                               and
                                                                                                                                                                                                                                                                                       to
                                                                                                                                                                             geometric_center_xyz = ca.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                               CA
                                                                                                                                                                                                                                                                                                       specified
                                                                                                                                                                                                                                                                                      flag)
                                                                                                                          geometric
                                                                                                       geometric_center_z_coordinate_CA(selection):
                                                                                                                                                                                                                                                                                                                                                                                                                  name
                                                                                                                                                            = selection.selectAtoms("name CA")
                                                                                                                                                                                                                                                                                      helices (based on 'system_name'
                                                                                                                                                                                                                                                                     the
                                                                                                                                                                                                                                                                                                         ಡ
                                                                                                                           the
                                                                                                                                                                                                                                                                                                                                                                                                                  II
                                                                                                                                                                                                                                                                                                                                                            for helix
                                                                                                                                                                                                                                                                    οĮ
                                                                                                                                                                                               geometric_center_xyz[2]
                                                                                                                                                                                                                                                                                                       gnuplot-ready output file for
                                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA_selection
                                                                                                                                                                                                                                                                    '''Prints the Z coordinate
  extracting
                                                                                                                          coordinate of
                                                                                                                                                                                                                                                                                                                                                            coordinate motion
                                                                                                                                                                                                                                                                                                                                                                            center helix 1).'''
                                                                                                                                         selection.'''
  for
                                                                                                                                                                                                                                                                                                                                                                                              system_name
''' MDAnalysis tools
                                   MDAnalysis
                                                    numpy
                                    import
                                                                       import
                                                                                                                                                                                                                                  def
                                                                                                                                                                                                                                       13
                                                                                                                                                                                                   11
                                                                                                                                                                                                                                                                                                           15
                                                                                                                                                                                                                                                                                                                                                                                 17
                                                                                                                                                                                                                                                                                                                                                                                                                    19
                                                                                                                                                                  356
```

```
output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         dimer trajectories.'''
                                                                                                                                                                                                               = universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                                    = universe_object.selectAtoms(helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                            using
                                                                                               second
                                                                                                                                                       Ν
  second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 (degrees) relative to bilayer normal (eigenvector approach) | | \n#column
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             || column 2: helix 1 tilt
                                                         first
                                                                                                                                                         center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     normal
                                                                                                                                                                                                                                                                                                                                                                                                                              normal
                                                                                                                                                                                                                                                                                          str(helix_1_CA_center_Z)
 #select
                                                                                               #select
                                                          #select
                                                                                                                                                                          coordinate \n')
                                                                                                                                                                                                                                                                                                                                                                                         skip_frames,
                                                                                                                                                     Z_outfile.write('#column 1: frame # || column 2: helix 1 geometric
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   and bilayer
                                                                                                                                                                                                                                                                                                                                                                                                                             bilayer
                                                                                                                                                                                                                                                                                                                                str(ts.frame)
34:66 )"
                                                                                              24:46 )"
                                                         =
                                                                                                                                                                                                                                                                                                                                                                                                                               t o
                                                         1:23
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         deal with FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                             (CA backbone) relative
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   eigenvector)
                                                                                                                                                                                                                                                                                                                                                                                       def helix_tilt_vs_bilayer_normal(folder_name, universe_object,
                                                                                                                                                                         Z
                                                                                                                                                                                             in universe_object.trajectory[::skip_frames]:
 and resid
                                                        resid
                                                                                              resid
                                                                                                                                                                       coordinate || column 3: helix 2 geometric center
                                                                                                                                                                                                                                                                                                                                -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frame #
                                                          and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_tilt_angle_file = open(output_file, 'w')
                                                                                                                                                                                                                                                                                        Z_outfile.write(str(ts.frame) + ' ' +
                                                                                               and
                                                                                                                                                                                                                                                                                                           str(helix_2_CA_center_Z) + ' n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    (first
CA
                                                         CA
                                                                                              CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         has been extended to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_tilt_angle_file.write('#column 1:
   name
                                                           name
                                                                                                name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    axis
                                                                                                                                   = open(output_file, 'w')
                                                                                              _
=
                                                                                                                                                                                                                                 centerOfGeometry()[2]
                                                                                                                                                                                                                                                                                                                                print str(folder_name) +
                                                                                                                                                                                                                                                                     .centerOfGeometry()[2]
                                                                                                                                                                                                                                                                                                                                                                                                                              helix
   II
                                                                                                II
                                                            II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   SVD technique to define helix
                                                                                               helix_2_CA_selection
helix_2_CA_selection
                                                         helix_1_CA_selection
                                                                                                                                                                                                                                                    helix_2_CA_center_Z
                                                                                                                                                                                                              helix_1_CA_center_Z
                                    == 'GpA':
                                                                                                                                                                                                                                                                                                                                                                                                                              of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      third eigevector). This
                    FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                              tilt
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          function
                                                                                                                                                                                                                                                                                                                                                                                                                              the
                                                                               monomer
                                                                                                                                                                                                                                                                                                                                                 Z_outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                   algebra
                                        system_name
                                                                                                                                                                                                                                                                                                                                                                                                                           '''Calculates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         particular
                                                                                                                                    Z_outfile
                                                                                                                                                                                                                                                                     357
                                                                                                                                                                                                                                                                                                                                                                                             33
                                                                                                   23
                                                                                                                                                            22
                                                                                                                                                                                                                                                                                                                                                        31
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        35
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                37
                                          ^{21}
                                                                                                                                                                                                                   27
```

```
2 tilt angle (degrees) relative to bilayer normal (eigenvector approach
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 geometric center coordinate value from the coordinates of the system
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 substract the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 eigenvector; checking in VMD
                                                                                                                                                                                                                                                                                                 helix_1_CA_selection = universe_object.selectAtoms("name CA and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA_geometric_center = helix_1_CA_selection.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_CA_geometric_center = helix_2_CA_selection.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               bilayer_phosphate_selection = universe_object.selectAtoms("name PO4")
                                                                                                                                                                                                       instead
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 phosphate_coordinates = bilayer_phosphate_selection.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = universe_object.selectAtoms("name CA
                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA_coordinates = helix_1_CA_selection.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_2_CA_coordinates = helix_2_CA_selection.coordinates()
                                                                                                                                                   #removed many of the comments used in monomer version, and
                                                                                                                                                                                               simple adjustments to select and deal with two monomers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               a SVD on the centered coordinates; so
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              phosphate_geometric_center = bilayer_phosphate_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             uu, dd, vv = numpy.linalg.svd(helix_1_CA_coordinates
                                                                                            in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_1_vector = vv[0] #this is the first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #repeat for second helix in dimer sims:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  it looks right along the helix axis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #when you call the numpy SVD function:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_1_CA_geometric_center)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #the idea is to do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             34:66")
                                                                                                                                                                                                                                                                                                                                           1:33")
helix
                                                                                                   for ts
```

358 [‡] 49

51

53

52

22

39

41

43

```
(phosphates
                                                                                                                               numpy.linalg.svd(bilayer_phosphate_selection.coordinates()
                                                                                                                                                                                      seems
                                                                                                                                                                                                                                                                on the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        helix_2_angle = math.acos(numpy.dot(helix_2_vector,bilayer_normal))
                                                                                                                                                                                                                                                                                                                helix_1_angle = math.acos(numpy.dot(helix_1_vector,bilayer_normal))
                                                                                                                                                                              = cc[2] #check in VMD: yes, the third eigenvector
                                                                                                                                                                                                         reasonable vector up +Z axis for bilayer normal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     def GpA_helix_tilt_vs_bilayer_normal(folder_name, universe_object, skip_frames
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_tilt_angle_file.write(str(ts.frame) + ' ' + str(theta_1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      gnuplot-ready columns
                                                                                                                                                                                                                                                               #angle between helix axis and bilayer normal vectors depends
                                                                             bilayer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   str(ts.frame)
uu, dd, vv = numpy.linalg.svd(helix_2_CA_coordinates
                                                                              the
                                                                                                                                                        -bilayer_phosphate_selection.centerOfGeometry())
                                                                              for
                                                                                process
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      str(theta_2) + '\n') #formatting for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  -- frame: '
                                                                                                                                                                                                                                                                                                                                                                    theta_1 = math.degrees(helix_1_angle)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           theta_2 = math.degrees(helix_2_angle)
                                                                              calculation
                        helix_2_CA_geometric_center)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = 180 - theta_2
                                                                                                                                                                                                                                                                                                                                                                                                                    = 180 - theta_1
                                                                             the eigenvector
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   print str(folder_name)
                                                 = vv [0]
                                                                                                                                                                                                                                                                                                                                        #convert to degrees:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #convert to degrees:
                                                                                                                                                                                                                                                                                                                                                                                          if theta_1 > 90:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    if theta_2 > 90:
                                                                                                                                                                                                                                                                                                                                                                                                                      theta_1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              theta_2
                                                                                                                                                                                                               ಡ
                                                                                                                                                                                 bilayer_normal
                                                    helix_2_vector
                                                                                                                                                                                                           produce
                                                                                                                                   II
                                                                                                                                                                                                                                                                                       product:
                                                                                                                               aa, bb, cc
                                                                              #repeat
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              output_file)
                                                           59
                                                                                                                                                                                                                                           63
                                                                                                                                                                                                                                                                                                                       65
                                                                                                                                                                                                                                                                                                                                                                         29
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 73
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   22
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       22
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    4
                                                                                                                                      61
                                                                                                                                                                                                                                                                                                                                                                                                                              69
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              71
                                                                                                                                                                                                                                                                                                                                                                     359
```

```
helix 2 tilt angle (degrees) relative to bilayer normal (eigenvector approach
                                                                                                                    helix_tilt_angle_file.write('#column 1: frame # || column 2: helix 1 tilt angle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       the coordinates of the system
  GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                substract the
                                                                                                                                                                                                                                                                                                                                                                     o f
                                                                                                                                                                                                                                                                                                                                                                                                                                                  and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    = universe_object.selectAtoms("name CA and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        = helix_2_CA_selection.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        bilayer_phosphate_selection = universe_object.selectAtoms("name PO4")
                                                                                                                                                           (degrees) relative to bilayer normal (eigenvector approach) | | \n # column 3:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_1_CA_geometric_center = helix_1_CA_selection.centerOfGeometry()
residue
                                                                                                                                                                                                                                                                                                                                                                     two monomers instead
                                                                                                                                                                                                                                                                                                                                  ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   phosphate_coordinates = bilayer_phosphate_selection.coordinates()
                                                                                                                                                                                                                                                                                                                              made
                                                                                                                                                                                                                                                                                                                                                                                                                                              = universe_object.selectAtoms("name CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_2_CA_coordinates = helix_2_CA_selection.coordinates()
  23
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_CA_coordinates = helix_1_CA_selection.coordinates()
                                                                                                                                                                                                                                                                                                                            version, and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            coordinates; so
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         bilayer_phosphate_selection.
tilt function to deal with
                                                                                                                                                                                                                                                                                                                          #removed many of the comments used in monomer
                                                                                                                                                                                                                                                                                                                                                                simple adjustments to select and deal with
                                                                                                                                                                                                                                                                               for ts in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     geometric center coordinate value from
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              a SVD on the centered
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #when you call the numpy SVD function:
                                                                           helix_tilt_angle_file = open(output_file, 'w')
'''Slightly altered version of helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       phosphate_geometric_center =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          helix_2_CA_geometric_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #the idea is to do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       24:46")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1:23")
                                                                                                                                                                                                                                        ('n/(
```

91

93

95

97

87

8 5 66

81

```
in VMD,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    dot
                                                                                                                                                                                                                                                        the eigenvector calculation process for the bilayer (phosphates
                                                                                                                                                                                                                                                                                                                      aa, bb, cc = numpy.linalg.svd(bilayer_phosphate_selection.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #angle between helix axis and bilayer normal vectors depends on the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      math.acos(numpy.dot(helix_2_vector,bilayer_normal))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_1_angle = math.acos(numpy.dot(helix_1_vector,bilayer_normal))
                                                                                                                                                                                                                                                                                                                                                                                   eigenvector
                                                                 eigenvector; checking
                                                                                                                                                                                                                                                                                                                                                                                                                 to produce a reasonable vector up +Z axis for bilayer normal
uu, dd, vv = numpy.linalg.svd(helix_1_CA_coordinates-
                                                                                                                                                         uu, dd, vv = numpy.linalg.svd(helix_2_CA_coordinates-
                                                                                                                                                                                                                                                                                                                                                                                 bilayer_normal = cc[2] #check in VMD: yes, the third
                                                                                                                                                                                                                                                                                                                                                   -bilayer_phosphate_selection.centerOfGeometry())
                                                              the first
                                                                                                                              #repeat for second helix in dimer sims:
                                                                                            it looks right along the helix axis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           theta_1 = math.degrees(helix_1_angle)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   theta_2 = math.degrees(helix_2_angle)
                                                            = vv[0] #this is
                                                                                                                                                                                         helix_2_CA_geometric_center)
                              helix_1_CA_geometric_center)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      = 180-theta_1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                theta_2 = 180 - theta_2
                                                                                                                                                                                                                         helix_2vector = vv[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #convert to degrees:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #convert to degrees:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   if theta_2 > 90:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        if theta_1 > 90:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_2angle =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        theta_1
                                                              helix_1_vector
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                product:
                                                                                                                                                                                                                                                        #repeat
                                                                                                                                                                                                                                                                                                                                                                                                                                                   361^{\frac{60}{10}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 113
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              115
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            117
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         119
                                                                      101
                                                                                                                                                                   103
                                                                                                                                                                                                                                                                105
                                                                                                                                                                                                                                                                                                                                                                                         107
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0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     try: #assigning an empty atom selection raises an exception,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_2\_selection\_string = "(around %s %s) and %s" % (str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix_1_selection_string = "( around %s %s ) and %s" % (str( boundary), helix_2_CA_selection, helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                  direction
                                                                                                                                                         helices in the
                                                                                                                                                                                                                          WOrk
                                                                                                                                                                                                                                                                                                                                                     closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          distance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      boundary), helix_1_CA_selection, helix_2_CA_selection)
   +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                universe_object
                                                                                                                           output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_2_residues_within_cutoff = universe_object
+ str(theta_1)
                         gnuplot-ready columns
                                                                                                                                                                                                                                                                                                                                                  || column 2:
                                                                                                                                                                                                                                                                                                                                                                                                                                                  any
                                                                                                                                                                                                                     given frame range and interval. This function has been confirmed
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          contacts are found within a given cutoff
                                                            str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                selectAtoms(helix_2_selection_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             selectAtoms(helix_1_selection_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                               boundary = 1 #start with contacts within 1 Angstrom in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_2_CA_selection = "( name CA and resid 24:46 )"
                                                                                                                         closest_approach_GpA(folder_name, universe_object, skip_frames,
                                                                                                                                                         '''Calculates the closest approach distance between the two
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_1_CA_selection = "( name CA and resid 1:23 )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_residues_within_cutoff =
                                                                                                                                                                                                                                                                                                                                                  number
                                                                                                                                                                                                                                                                                                                                                                                                             for ts in universe_object.trajectory[::skip_frames]:
helix_tilt_angle_file.write(str(ts.frame) +
                         str(theta_2) + ' n' = \#formatting for
                                                           -- frame: '
                                                                                                                                                                                                                                                                                                                                                  closest_approach_file.write('# column 1: frame
                                                                                                                                                                                                                                                                                                                   ( M |
                                                                                                                                                                                                                                                                                   purpose. '''
                                                                                                                                                                                                                                                                                                                     = open(output_file,
                                                                                                                                                                                                                                                                                                                                                                               helix-helix contact (Angstroms)\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   using a try block
                                                                                                                                                                                          simulations for
                                                                                                                                                                                                                                                     properly against my VMD tcl script
                                                         str(folder_name) +
                                                                                                                                                                                                                                                                                   that was designed for the same
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         distance_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       between helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          while 1: #until
                                                                                                                                                                                                                                                                                                                   closest_approach_file
                                                                                                                                                                                     control) GpA dimer
                                                           print
                                                                                                                                                                                                                             ಡ
                                                                                                                           def
                                                                                                                                125
                                                                                                                                                                                                                                                                                                                                                                                                                                                 362^{\frac{c}{c}}
                                                                   123
                                                                                                                                                                                                                             127
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        141
                                                                                                                                                                                                                                                                                                                           129
                                                                                                                                                                                                                                                                                                                                                                                                                       131
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    135
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               137
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           139
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363

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acceptable
                                                                                                                                                                                                                                                                                                                              = "( name CA and resid 24:46 )" #select second GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               there are no
                                                                                                                                                                                                                                                                    CA and resid 1:23 )" #select first GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_2_residues_within_cutoff = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              atoms in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      distance from helix 1 set above. If there are contacts, no
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    exception will be raised and the function can move on to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ranking the closest contacts by residue name and number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         contacts within the helix_proximity_requirement
occurence in top
                                                                                                                                                                                  a new trajectory,
                                                                                                                                                 each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            CA
                                                         within this number
                                                                                                                                                                                                                                                                                                                                                                                        = "( around %s %s ) and %s" % (str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              close enough select all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_2_selection_string) #raises Exception if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_2_CA_residues = universe_object.selectAtoms
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_1_CA_residues = universe_object.selectAtoms
                                                                                                                                                   contacts from
                                                                                                                                                                                                                                                                                                                                                                                                                         helix_proximity_requirement), helix_1_CA_selection,
and normalized frequency of
                                                                                                                                                                                 οĮ
                                                                                                                                                                                 start
                                                                                                                                                                                                                                        in universe_object.trajectory[::skip_frames]:
                                                           рe
                                                                                                                                                   closest
                                                           must
                                                                                                                                                                               the
                                                                                                                                                                               frame will be appended here; resets at
                                                                                                                                                compound_distance_list = [] #the top five
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 are
                                                         # helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_2_CA_selection)
                                                                                      Angstroms for contacts to be counted
                                                                                                                                                                                                                                                                 "( name
                                                                                                                      top_five_file = open(output_file, 'w')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #Now that the helices
                                                                                                                                                                                                                                                                                                                                                                                        helix_2_selection_string
                                                        helix_proximity_requirement = 7
                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_2_CA_selection)
                                                                                                                                                                                                                                                                    helix_1_CA_selection =
                           contacts on the y-axis.'''
                                                                                                                                                                                                                                                                                                                              helix_2_CA_selection
N->C residue name on x-axis
                                                                                                                                                                                                                                                                                                                                                                  monomer
                                                                                                                                                                                                                                                                                                         monomer
                                                                                                                                                                                                              simulation
       161
                                                                                                                                                                                                                                                                                                                                       ^{167}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               173
                                                                                                                            163
                                                                                                                                                                                                                                                 165
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            169
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       171
```

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at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_1_merged_list = zip(helix_1_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_2_merged_list = zip(helix_2_ordered_residue_names_numbers
                                                                                                                                                        residue
                                                                                                                                                                                                                                                                                                                                                                        helix_1_identifiers[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     and numbers (in tuples
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       their respective
                                                                                         helix_2_CA_residues.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               = [(residue.resname,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_2_ordered_residue_names_numbers = [(residue.resname,
                                                          = helix_1_CA_residues.coordinates()
                                                                                                                                                   generate a list of ordered
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          residue.resid) for residue in helix_2_identifiers]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             in helix_1_identifiers]
ordered
                                                                                                                                                                                  identifiers (MDAnalysis format) for each helix:
                                                                                                                                                                                                                  helix_1_identifiers = list(helix_1_CA_residues)
                                                                                                                                                                                                                                                = list(helix_2_CA_residues)
o f
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       numbers with
lists
                                                                                                                                                                                                                                                                                                                                                                       #print helix_1_identifiers[0].resname,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     ordered residue names
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_1_ordered_residue_names_numbers
generate
                                                                                                                                                                                                                                                                                                           #test output format at terminal:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #now zip the residue names and
                            coordinates for each helix:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for residue
                                                                                                                                                                                                                                                                                                                                           #print helix_1_CA_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_1_CA_coordinates)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_CA_coordinates)
                                                                                                                                                     #Use the atomselections to
atomselections to
                                                                                            II
                                                                                         helix_2_CA_coordinates
                                                           helix_1_CA_coordinates
                                                                                                                                                                                                                                                 helix_2_identifiers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               residue.resid)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #make a list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   the moment)
#Use the
                                                                                                                                                                                                                                                                                                                                                                                                        resid
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 $365^{\frac{1}{28}}$

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```
and
 in helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               contacts between
                                                                                                                                                                                                                                                                                                            (helix_2_CA, coordinate_2) in helix_2_merged_list:
                                                                                                                                                                                                         that
                                                                                                                                                        distance
                                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA, helix_2_CA, measured_distance])
                                                                                                                              interactions
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      given frame to an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                #sort the list of labelled distances by the 'third element,'
                                                   element would be the corresponding residue name
                                                                                                                                                                                                                                                                                                                                    measured_distance = numpy.linalg.norm(numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        are close enough)
                                                                                                                                                                                                        #between the CA particles which retains the residue names
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   element:
                                                                                                                                                                                                                                                                                                                                                             subtract(coordinate_1, coordinate_2))
 of the 23rd CA
                                                                                                                                                                                                                                                                                for (helix_1_CA, coordinate_1) in helix_1_merged_list:
                                                                                                                                                                                                                                                                                                                                                                                     labelled_interhelix_distances.append([
                                                                                                                                                        the
                                                                                                                                                       list of the
                                                                                                                              residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          element in labelled_interhelix_distances[0:5]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 labelled_interhelix_distances.sort(key = lambda
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          which is the distance between CA particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #append the five closest contacts from this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      given frame (if they
                                                                                                                              οĘ
 coordinate
                        helix_1_merged_list[22][1]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ascending list of
                                                                                                                              combinations
                                                                                                                                                         ಡ
                                                                                                                                                        make
                                                                                                                                                                                                                                                         correspond to the interaction
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   frame:
                                                                                                                                                        between the two helices and
                                                                                                                                                                                                                                                          labelled_interhelix_distances
give the [x y z]
                                                                                                                            possible
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helices for this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 overall list for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #so, now there is
                                                                                                                             #go through all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             element[2])
                                                                                                                                                                              magnitude)
                         print
                                                                                                                                                                                                                                                                                                             for
 would
                                                  #the zeroth
                                                                             number
  #this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for
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215	<pre>compound_distance_list.append(element) #still contains residue 1 and 2 names along with distance</pre>
	<pre>except Exception: #the helices aren't close enough for a meaningful interface residue assessment defined by helix_proximity_requirement above</pre>
217	pass #so just leave the try block and jump to the next frame iteration in the for loop
219	<pre>print str(folder_name) + ' frame: ' + str(ts.frame)</pre>
221	#the compound distance list should now contain all the ton five contacts
$\frac{\tilde{\tilde{s}}}{367}$	ncatenated from all eligible frames for the current trajectors to go through this list and the number of times each pair vide by the total number of pairs
225	<pre>#determine number of top five contacts in total: num_pairs = len(compound_distance_list)</pre>
227	helix_1_partner_list = [] helix_2_partner_list = []
231	lists with each of the rse contacts from the restriner, helix_2_partner,
233	helix_1_partner_list.append(helix_1_partner) #the involved residue from helix 1 gets appended helix_2_partner_list.append(helix_2_partner) #same for cognate helix_2
	residue in a separate list

```
= helix_2_partner_list.count(residue2_name) #raw residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     could be
 showed
                                                                                           important
                                                                                                                                                                                                                                                                                                                                                                 file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 unique
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  picked
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #raw
                                                         so can use the 'set' data structure to remove
                                                                                                                                                                                                                                                                                                                                                                                                                         resname || resnum |
each list basically has an entry for every time a given residue
                                                                                                                                                                                                                                                                                                                                                                  o f
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_2_no_duplicates): #limited by the shortest list though; this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        a given residue from a given helix, and print result
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      resname || resnum
                                                                                                                                                                                                                                                                                                                                                                 top
                                                                                         the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             = helix_1_partner_list.count(residue1_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  a really small number of unique residues were
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             all residues counted by frame and count
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for (residue1_name, residue2_name) in zip(helix_1_no_duplicates,
                                                                                                                                                                                                                                                                                                                                                                  at
                                                                                      duplicates for iteration purposes without actually removing
                              the
                                                                                                                                                                                                                                                                                                                                                               specified
                            the top five for an acceptable frame from one of
                                                                                                                                                                                                                                                                                                    #top_five_file.write(str(helix_1_no_duplicates)+'\n')
                                                                                                                                                                                                                                                                       #top_five_file.write(str(helix_1_partner_list)+'\n')
                                                                                                                                                                                                                                                                                                                                                                                                                          helix 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    N
                                                                                                                                                                                                                                                                                                                                                                 format,
                                                                                                                                                  = set(helix_1_partner_list)
                                                                                                                                                                                set(helix_2_partner_list)
                                                                                                                                                                                                                                          #test print compare the two to verify duplicate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   top_five_file.write('#last three columns: helix
                                                                                                                      #multiple occurences within the lists proper
                                                                                                                                                                                                                                                                                                                                                                                                                         columns:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frequency in top 5 closest contacts \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                    contacts \n')
                                                                                                                                                                                                                                                                                                                                                              following
                                                                                                                                                                                                                                                                                                                                                                                                                          three
                                                                                                                                                                                                                                                                                                                                                               the
                                                                                                                                                                                                                                                                                                                                                                                                                                                      5 closest
                                                                                                                                                                                                                                                                                                                                                               be printed in
                                                                                                                                                                                                                                                                                                                                                                                                                         top_five_file.write('#first
                                                           #This includes duplicates
                                                                                                                                                                                                                                                                                                                                                                                              gnuplot-friendly form:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #go through the list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            residue1_count
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       counts for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        residue2_count
                                                                                                                                                   helix_1_no_duplicates
                                                                                                                                                                                helix_2_no_duplicates
                                                                                                                                                                                                                                                                                                                                                                                                                                                      in top
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   problem if only
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         occurence of
                                                                                                                                                                                                                                                                                                                                                               #results will
                                                                                                                                                                                                                                                                                                                                                                                                                                                         frequency
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```
#because we
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               acceptable
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CA and resid 1:33 )" #select first FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                                  FGFR3
                                                                                                                            47
                                                                                                                                                                                                                                 +
                                                                                                                                                                                                             1ue2_name[0]).strip("'") + ' ' +str(residue2_name[1]+(72-23))
str(residue2_frequency) + '\n')
                                                                                                                                                                                                   str(residue1_frequency) + ' ' + str(
                                                                                                                            2008 vol.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                "(name CA and resid 34:66)" #select second
                                                                                                                                                                         top_five_file.write(str(residue1_name[0]).strip("'") + ' ' +str(
                                                                                                    residue
                                                                                                                                                                                                                                                                                                                                                                                                                                  adjusted to work for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        a new trajectory
                                                                                                                                                                                                                                                                                                                                                                                  universe_object, skip_frames
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             contacts from each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     within this number
 = float(residue1_count)/float(num_pairs)
                                                = float(residue2_count)/float(num_pairs)
                                                                                                                           to match those use in Emi's work (Biochemistry
                                                                                                   for
                                                                                                    corrected
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        οĮ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       start
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                   similarly named function
                                                                                                  that are simultaneously
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     must be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     the
                         normalized frequency'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     frame will be appended here; resets at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           compound_distance_list = [] #the top five
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_proximity_requirement = 7 # helices
                                                                                                                                                                                                                                                                                                                                                                                 def top_five_closest_residues_FGFR3(folder_name,
                                                                                                                                                                                                                         residue2_name[0]).strip("'")
                                                                                                                                                                                                   residue1_name[1]+72) + ' ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Angstroms for contacts to be counted
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          "( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     top_five_file = open(output_file, 'w')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_2_CA_selection =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_CA_selection
                                                                                                    #print out results
                                                                                                                                                                                                                                                                                                                                                                                                                                  of the
residue1_frequency
                                                residue2_frequency
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         FGFR3 monomer
                          the
                                                                                                                                                                                                                                                                                                                              top_five_file.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                  '''Modified version
                                                                                                                              numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               simulation
                                                                                                                                                                                                                                                                                                                                                                                                           output_file):
                                                                                                                                                                                                                                                                                                                                                          369
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     269
                                                        255
                                                                                                                                                                                                                                                                                    259
                                                                                                                                                                                                                                                                                                                                      261
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          265
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  267
                                                                                                         257
```

```
there are no
                                                                                                                                             helix_2_residues_within_cutoff = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                        atoms in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    residue
                                                                                                                                                                                                                                                        distance from helix 1 set above. If there are contacts, no
                                                                                                                                                                                                                                                                                           exception will be raised and the function can move on to
                                                                                                                                                                                                                                                                                                                                  ranking the closest contacts by residue name and number.
                                                                                                                                                                                                                     contacts within the helix_proximity_requirement
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_1_CA_coordinates = helix_1_CA_residues.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        helix_2_CA_residues.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               generate a list of ordered
                                                                                                                                                                                                                                                                                                                                                                                                        CA
   (str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #Use the atomselections to generate lists of ordered
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix_2_CA_residues = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  identifiers (MDAnalysis format) for each helix:
                                                                                                                                                                                                                                                                                                                                                                                                        #Now that the helices are close enough select all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_CA_residues = universe_object.selectAtoms
                                                                                                                                                                                  helix_2_selection_string) #raises Exception if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          helix_1_identifiers = list(helix_1_CA_residues)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_identifiers = list(helix_2_CA_residues)
= "( around %s %s ) and %s" %
                                      helix_proximity_requirement), helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #test output format at terminal:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  coordinates for each helix:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #print helix_1_CA_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #Use the atomselections to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_2_CA_coordinates =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_2_CA_selection)
 helix_2_selection_string
                                                                       helix_2_CA_selection)
                                                                                                              try:
         271
                                                                                                                                                       273
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     370
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      285
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            287
                                                                                                                                                                                                                                                                                                                                                                                                                275
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          279
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           283
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   281
```

```
at
                                                                                                                                                                                                                                              CA
                                                                                                                                                                                                                                                                                                                                                                                                                  of the 23rd CA in helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 element would be the corresponding residue name and
                                                                                                                                                                                                                                                                                           helix_1_merged_list = zip(helix_1_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                          helix_2_merged_list = zip(helix_2_ordered_residue_names_numbers
helix_1_identifiers[0].
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   that
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              (helix_2_CA, coordinate_2) in helix_2_merged_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   distance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          interactions
                                                                        tuples
                                                                                                                                                                                                                                              #now zip the residue names and numbers with their respective
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 residue names
                                                                                                                                                                     helix_2_ordered_residue_names_numbers = [(residue.resname
                                                                                                                       = [(residue.resname
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     for (helix_1_CA, coordinate_1) in helix_1_merged_list:
                                                                         (in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   the
                                                                                                                                              in helix_1_identifiers]
                                                                                                                                                                                              residue.resid) for residue in helix_2_identifiers]
                                                                        numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #between the CA particles which retains the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 list
                                                                        and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          o f
                                                                                                                                                                                                                                                                                                                                                                                                                   coordinate
#print helix_1_identifiers[0].resname,
                                                                                                                                                                                                                                                                                                                                                                                                                                         1: print helix_1_merged_list[22][1]
                                                                                                                       helix_1_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          combinations
                                                                          names
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   make a
                                                                         residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       correspond to the interaction
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                labelled_interhelix_distances =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   and
                                                                                                                                               for residue
                                                                                                                                                                                                                                                                                                                                                                                                                   #this would give the [x y z]
                                                                                                                                                                                                                                                                                                                                                                    helix_2_CA_coordinates)
                                                                                                                                                                                                                                                                                                                    helix_1_CA_coordinates)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   the two helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         possible
                                                                         ordered
                                                                                                                                              residue.resid)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #go through all
                                                                        #make a list of
                                                                                                                                                                                                                                                                       coordinates
                                                                                                 the moment)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       magnitude)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #the zeroth
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    between
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           number
                           resid
      289
                                                                                                                                                                                                                                                                                                                                              $\\ 371
                                                                                                                                                                            293
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       303
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              305
                                                                                                                                                                                                                                                    295
                                                                              291
                                                                                                                                                                                                                                                                                                                                                                                                                          299
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        301
```

	#want to go through this list and the number of times each pair shows up and divide by the total number of pairs
327	umber of top five collen(compound_distance
331	_ st st
333	helix_2_partner_list = [] #make separate lists with each of the residue names (including duplicates) found in close contacts from the respective helices
33 52	partner, helix_2_partner, separation) ix_1_partner_list.append(helix_1_partner
	helix 1 gets appended helix_2_partner) #same for cognate helix 2
373 \$	n a separate list
	#Now, each list basically has an entry for every time a given residue showed up in the top five for an acceptable frame from one of the helices
339	s duplicates so can use the 'set' data structure to re
	nicates for iteration purposes without ple occurences within the lists proper
341	helix_1_no_duplicates = set(helix_1_partner_list) helix_2_no_duplicates = set(helix_2_partner_list)
343	
8. 5.45	<pre>#test print compare the two to verify duplicate removal: #top five file.write(str(helix 1 partner list)+'\n')</pre>
	five_file.write(str(helix_1_no_duplic
347	
	#results will be printed in the following format, specified at top of file in gnuplot-friendly form:

```
residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                    We
                                                                                                                                                                                                                                                                                                           residue
                                                                                                                                                                                                                        helix_2_no_duplicates): #limited by the shortest list though; this could be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 residue2_name[0]).strip("'") + ' ' +str(residue2_name[1]+(366-33))
                                                                                                                                                                                                                                                 picked from
                                                                                                                                                                                                                                                                                                                                                                                                                                             residue1_frequency = float(residue1_count)/float(num_pairs) #because
                                                                                                                                                                    to file
                                                                                                                                       unique
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                +str(
                                                                                                                                                                                                                                                                                                                                                               #raw
                                                                                                                                                                                                                                                                                                         #raw
helix 1 resname || resnum ||
                                                                                                                                                               given helix, and print result
                                                                                                                                        the
                                                     columns: helix 2 resname || resnum
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    = float(residue2_count)/float(num_pairs)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        + ' + str(residue1_frequency) +
                                                                                                                                                                                                                                                                                                         = helix_1_partner_list.count(residue1_name)
                                                                                                                                                                                                                                                                                                                                                              = helix_2_partner_list.count(residue2_name)
                                                                                                                                                                                                                                                  a really small number of unique residues were
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              top_five_file.write(str(residue1_name[0]).strip("'") + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           for
                                                                                                                                          count
                                                                                                                                                                                         for (residue1_name, residue2_name) in zip(helix_1_no_duplicates,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #print out results that are simultaneously corrected
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      numbers to match those used in FGFR3 literature
                                                                                                                                       counted by frame and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               str(residue2\_frequency) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         want the 'normalized frequency'
top_five_file.write('#first three columns:
                                                                                contacts \n')
                                                                                                                                                                   a given residue from a
                             5 closest contacts
                                                                                                                                       residues
                                                       three
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          residue1_name[1]+366)
                                                                                  5 closest
                                                                                                                                                                                                                                                                                                                                                                                           helix2
                                                                                                                                                                                                                                                                                                                                     helix1
                                                                                                                                     a11
                                                      top_five_file.write('#last
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    residue2_frequency
                                                                                                                                       #go through the list of
                                                                                                                                                                                                                                                                                                          residue1_count
                                                                                                                                                                                                                                                                                                                                   counts for
                                                                                                                                                                                                                                                                                                                                                               residue2_count
                                                                                                                                                                                                                                                                                                                                                                                           counts for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 top_five_file.close()
                                                                                top
                             in top
                                                                                                                                                                                                                                                     problem if only
                                                                                   in
                                                                                                                                                                   Jo
                                frequency
                                                                                   frequency
        349
```

374

357

359

361

+

363

365

ಡ

351

GFR3 (mutant_overall verse_object, skip_ merges the 'top fi mers into separate rsed and compared in es.'''	Angstroms for contacts to be counted compound_distance_list = [] #the top five closest contacts from each acceptable frame will be appended here; resets at the start of a new trajectory/ simulation	<pre>for ts in universe_object.trajectory[::skip_frames]: helix_1_CA_selection = "(name CA and resid 1:33)" #select first FGFR3</pre>	monomer helix_2_CA_selection = "(name CA and resid 34:66)" #select second FGFR3 monomer	<pre>helix_2_selection_string = "(around %s %s) and %s" % (str(</pre>	helix_2_residues_within_cutoff = universe_object.selectAtoms(helix_2_selection_string) #raises Exception if there are no helix 2 contacts within the helix_proximity_requirement distance from helix 1 set above. If there are contacts, no exception will be raised and the function can move on to ranking the closest contacts by residue name and number.	#Now that the helices are close enough select all CA atoms in each:
367	371	373	375	375	377	379

```
#now zip the residue names and numbers with their respective CA
                                                                                                                                                                                                                                                                                                      #Use the atomselections to generate a list of ordered residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #print helix_1_identifiers[0].resname, helix_1_identifiers[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             tuples
                                                                                                                                                                                                              helix_1_CA_coordinates = helix_1_CA_residues.coordinates()
                                                                                                                                                                                                                                         helix_2_CA_coordinates = helix_2_CA_residues.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_2_ordered_residue_names_numbers = [(residue.resname
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helix_1_ordered_residue_names_numbers = [(residue.resname
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             (in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_1_identifiers]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              residue.resid) for residue in helix_2_identifiers]
                                                                                                                                                    ordered
helix_1_CA_residues = universe_object.selectAtoms(
                                                           = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             and numbers
                                                                                                                                                                                                                                                                                                                                  identifiers (MDAnalysis format) for each helix:
                                                                                                                                                                                                                                                                                                                                                                helix_1_identifiers = list(helix_1_CA_residues)
                                                                                                                                                                                                                                                                                                                                                                                             helix_2_identifiers = list(helix_2_CA_residues)
                                                                                                                                                    Jo
                                                                                                                                                  lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               names
                                                                                                                                                  generate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      in
                                                                                                                                                                                                                                                                                                                                                                                                                                                         #test output format at terminal:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             residue
                                                                                                                                                                               coordinates for each helix:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    for residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #print helix_1_CA_coordinates
                                                                                                                                                  #Use the atomselections to
                                                                                     helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ordered
                         helix_1_CA_selection)
                                                      helix_2_CA_residues
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   residue.resid)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #make a list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            the moment)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  399
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         397
                                                                   381
                                                                                                                                                          383
                                                                                                                                                                                                                                                  385
                                                                                                                                                                                                                                                                                                              387
                                                                                                                                                                                                                                                                                                                                                                                                     389
                                                                                                                                                                                                                                                                                                                                                                                                                              376^{\frac{7}{6}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   395
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            393
```

at

```
in helix
                                                                                                                                                                                              #the zeroth element would be the corresponding residue name and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            ascending list of closest contacts between
helix_1_merged_list = zip(helix_1_ordered_residue_names_numbers
                                                    helix_2_merged_list = zip(helix_2_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 for (helix_2_CA, coordinate_2) in helix_2_merged_list:
                                                                                                                                                                                                                                                                                                                                                                       that
                                                                                                                                                                                                                                                                                                                distance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA, helix_2_CA, measured_distance])
                                                                                                                                                                                                                                                                                   of residue interactions
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      element,'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               = numpy.linalg.norm(numpy
                                                                                                                                                                                                                                                                                                                                                                     residue names
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              element:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         subtract(coordinate_1, coordinate_2))
                                                                                                                                           CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                    for (helix_1_CA, coordinate_1) in helix_1_merged_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  labelled_interhelix_distances.append([
                                                                                                                                                                                                                                                                                                                the
                                                                                                                                           the 23rd
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     of labelled distances by the 'third
                                                                                                                                                                                                                                                                                                                the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            labelled_interhelix_distances.sort(key = lambda
                                                                                                                                                                                                                                                                                                              οĘ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                which is the distance between CA particles
                                                                                                                                                                                                                                                                                                                                                                     retains the
                                                                                                                                           οĘ
                                                                                                                                                                                                                                                                                                              list
                                                                                                                                           coordinate
                                                                                                                                                                   1: print helix_1_merged_list[22][1]
                                                                                                                                                                                                                                                                                  #go through all possible combinations
                                                                                                                                                                                                                                                                                                              and make a
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              measured_distance
                                                                                                                                                                                                                                                                                                                                                                    #between the CA particles which
                                                                                                                                                                                                                                                                                                                                                                                                                           labelled_interhelix_distances =
                                                                                                                                                                                                                                                                                                                                                                                                correspond to the interaction
                                                                                                                                      give the [x y z]
                                                                                   helix_2_CA_coordinates)
                             helix_1_CA_coordinates)
                                                                                                                                                                                                                                                                                                              between the two helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #so, now there is an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #sort the list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         element[2]
                                                                                                                                                                                                                                                                                                                                         magnitude)
                                                                                                                                         #this would
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      417
                                                                                                                                                                                                                                                              405
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 413
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     415
                                                               401
                                                                                                                                                403
                                                                                                                                                                                                                                                                                                                                                                             407
                                                                                                                                                                                                                                                                                                                                                                                                                                                               409
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     411
```

are close enough)

given frame (if they

helices for this

```
contains
                                                                                                                                                                        defined by helix_proximity_requirement
      an
                                                                                                                                                                                                                          frame
                                                                                                                                                                                                                                                                                                                                                                                                                         want
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       in
                                                                                                                                                 except Exception: #the helices aren't close enough for a meaningful
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #list defined in merged_distance_lists() function above (which is called
                                                                                                                                                                                                                                                                                                                                                                      #the compound_distance_list should now contain all the top five contacts
 frame
                                                                                                                                                                                                                        pass #so just leave the try block and jump to the next
                                                                                                                                                                                                                                                                                                                                                                                                                       I will
                                                                         compound_distance_list.append(element) #still
                                                                                                   along with distance
                                                                                                                                                                                                                                                                                                                                                                                                concatenated from all eligible frames for the current trajectory
given
                                                element in labelled_interhelix_distances[0:5]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                to parse the trajectory
                                                                                                                                                                                                                                                                                                                          str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                    merged approach is that
                                                                                                                                                                                                                                                                                                                                                                                                                                                 simply dump the compound_distance_list to the appropriate
  this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                hetero_overall_list.append(element)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      mutant_overall_list.append(element)
  from
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       element in compound_distance_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            compound_distance_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         are ready
  contacts
                                                                                                                                                                                                                                                                                                                          +
                                                                                                  2 names
                          frame:
                                                                                                                                                                                                                                                                                                                          frame:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              elif 'wildtype_dimer' in folder_name:
                                                                                                                                                                                                                                               loop
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       elif 'mutant_dimer' in folder_name:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        conditions/lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                another function
                                                                                                  and
  the five closest
                          each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               in folder_name:
                                                                                                                                                                                                                                                                                                                                                                                                                       different with this
                                                                                                                                                                                                                                                for
                                                                                                                                                                          residue assessment
                                                                                                  residue 1
                        overall list for
                                                                                                                                                                                                                                                iteration in the
                                                                                                                                                                                                                                                                                                                          +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                element in
                                                                                                                                                                                                                                                                                                                        str(folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #after each of the three
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               if 'heterodimer'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                main()), and then use
  #append
                                                                                                                                                                           interface
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for
                                                  for
                                                                                                                                                                                                                                                                                                                                                                                                                        #**the part that is
                                                                                                                                                                                                                                                                                                                        print
        419
                                                                                                                                                                                                                                                                                                                          \frac{1}{2}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     437
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     439
                                                                                                                                                       423
                                                                                                                                                                                                                                                                              425
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             431
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     433
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      435
                                                                               421
                                                                                                                                                                                                                                                                                                                                                                               429
```

```
helix_2_partner_list.append(helix_2_partner) #same for cognate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     dimer_batch_analysis_symlink/merged_top_five_data/%s_merged_topfive
                                                                                                                                                                       the
                                                                                                                                                                                                                                                                                                                                                                                                                                                  the work
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          helix_1_partner_list.append(helix_1_partner) #the involved
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           duplicates) found in close contacts from the respective helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           for (helix_1_partner, helix_2_partner, separation) in input_list:
                                                                                                                                                                          J O
                                                                                                  def parse_overall_FGFR3_top_five_data(mutant_overall_list,wildtype_overall_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #make separate lists with each of the residue names (including
                                                                                                                                                                       each
                                                                                                                                                                                                                                                                                                                                                                                                                                                  οĮ
                                                                                                                                                                     for
                                                                                                                                                                                                                                                                                                                                                                                                                                                most
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                file_path = '/sansom/sc2/bioc1009/Documents/FGFR3_work/
                                  wildtype_overall_list.append(element)
                                                                                                                                                                   analysis on merged 'top five contacts' data
                                                                                                                                                                                                                                                                                                                                                                                                                                              def parser_printer(input_list, condition, num_pairs): #does
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      open(str(file_path),'w')
element in compound_distance_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             residue from helix 1 gets appended
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix 2 residue in a separate list
                                                                                                                                                                                                                                                                         #determine number of top five contacts in total:
                                                                                                                                                                                                    print to file. "''
                                                                                                                                                                                                                                                                                                                                                                             num_wildtype_pairs = len(wildtype_overall_list)
                                                                                                                                                                                                                                                                                                        num_hetero_pairs = len(hetero_overall_list)
                                                                                                                                                                                                                                                                                                                                            = len(mutant_overall_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  a given list of contact pairs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   merged_top_five_outfile =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_1_partner_list =
                                                                                                                                                                                                       three FGFR3 conditions and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_2_partner_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    out' % condition
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 print input_list
   for
                                                                                                                                                                       ''Perform final
                                                                                                                                                                                                                                                                                                                                            num_mutant_pairs
                                                                                                                                      hetero_overall_list)
                                                                                                            443
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           461
                                           441
                                                                                                                                                                                                                                                 445
                                                                                                                                                                                                                                                                                                                  447
                                                                                                                                                                                                                                                                                                                                                                                     449
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               455
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 457
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      459
                                                                                                                                                                                                                                                                                                                                                                                                                                                         451
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            453
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  379
```

```
residue2_count = helix_2_partner_list.count(residue2_name) #raw
                                                                                                   to remove
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          a really small number of unique residues
    residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                    '#columns 4-6 (%s): helix 2 resname || resnum || frequency
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  count the
                                                                                                                                without actually removing the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          residue1_count = helix_1_partner_list.count(residue1_name)
                                                                                                                                                                                                                                                                                                                             top
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_no_duplicates): #limited by the shortest list though;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            for (residue1_name, residue2_name) in zip(helix_1_no_duplicates,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 and
given
                                                                                                   structure
                                an acceptable frame from one
                                                                                                                                                                                                                                                                                                                      #results will be printed in the following format, specified
                                                                                                                                                                                                                                                                                                                                                                                     resnum ||
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  all residues counted by frame and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               given residue from a given helix,
    entry for every time a
                                                                                               can use the 'set' data
                                                                                                                                                                                                                                                                                                                                                                                       '#columns 1-3 (%s): helix 1 resname ||
                                                                                                                                                                                                                            = set(helix_1_partner_list)
                                                                                                                                                                                                                                                         set(helix_2_partner_list)
                                                                                                                                                                                            #multiple occurences within the lists proper
                                                                                                                                                                                                                                                                                                                                                                                                                  closest contacts \n' % condition
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 in top 5 closest contacts \n' % condition
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   merged_top_five_outfile.write(string_1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  merged_top_five_outfile.write(string_2)
                                                                                                                                for iteration purposes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         residue counts for helix1
                                                                                                                                                                                                                                                                                                                                                        file in gnuplot-friendly form:
 #Now, each list basically has an
                                   the top five for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             only
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          were picked from one helix
                                                                                                  duplicates so
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          could be a problem if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ಡ
                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #go through the list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               unique occurence of
                                                                                                                                                                                                                            helix_1_no_duplicates
                                                                                                                                                                                                                                                           helix_2_no_duplicates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               result to file
                                                                                                #This includes
                                                                                                                                duplicates
                                                                                                                                                                                                                                                                                                                                                                                                                      in top 5
                                                                                                                                                                                                                                                                                                                                                                                          II
                                                                                                                                                                                                                                                                                                                                                                                                                                                      string_2 =
                                                                                                                                                                                                                                                                                                                                                                                       string_1
```

463

465

467

475

473

471

380

residue counts for helix2

```
residue1_frequency) + ' ' + str(residue2_name[0]).strip("'")
                                                                                                                                                                                                                    merged_top_five_outfile.write(str(residue1_name[0]).strip("'")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 to parse glycophorin A
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Basically subtract the coordinates of the geometric center of the reference
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ''Tracks the motion of one helix (its geometric center) relative to a helix
float(residue1_count)/float(num_pairs)
                                                                       = float(residue2_count)/float(num_pairs)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           is to produce something similar to Figure 4 in Biochemistry,
                                                                                                                                                                                residue numbers to match those used in FGFR3 literature
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          output_file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               with geometric center coordinates adjusted to (0,\ 0,\ 0) in each frame.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       data for
                                                                                                                                                simultaneously corrected for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               parser_printer(wildtype_overall_list, 'wildtype', num_wildtype_pairs)
                                                                                                                                                                                                                                                                                                                           + ' ' +str(residue2_name[1]+(366-33)) + ' ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   geometric centers -- its own and that of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'heterodimer', num_hetero_pairs)
                                                                                                                                                                                                                                                       + ' + str(residue1_name[1]+366) + ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     merged
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        def relative_helical_motion(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'mutant', num_mutant_pairs)
                                      the 'normalized frequency'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     οĮ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              2008, 10507. Accepts system name of 'GpA'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #now call the function to parse and print three files
                                                                                                                                                                                                                                                                                                                                                                residue2_frequency) + ' \setminus n')
                                                                                                                                                that are
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            merged_top_five_outfile.close()
         II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            parser_printer(mutant_overall_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           parser_printer(hetero_overall_list,
                                                                                                                                                results
                                    want
 residue1_frequency
                                                                       residue2_frequency
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      FGFR3 dimer condition simulated
                                          because we
                                                                                                                                               #print out
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        helix from both of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           . The idea
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                47, No.40,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               493
            479
                                                                                                                                                                                                                                                                                                                                                                                                                                                    485
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               381
                                                                                                                                                                                                                               483
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    489
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          491
                                                                                                                     481
```

```
triconv
                                                                                                                   FGFR3
                                                                                                                                                                                                                                                                                24:46 )" #select second GpA
those trajectories. Important note: ** this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    each
                                                                                                                                                                                                                                   1:23 )" #select first GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Will
                                                                                                                                                                  second
                                                                                                                                                                                                                                                                                                                                                     helix_tracking_file.write('#coordinate of geo center, format: ref helix (x)
                                                                                                                   first
                           in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #for the purpose of the plot we want to produce, helix 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix with geometric center at 0,0,0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_1\_center = helix_1\_CA\_residues.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_2 center = helix_2 CA_residues.centerOfGeometry()
                                               periodic boundary behaviour (avoid jumping from one
                        with -pbc nojump and -center used
                                                                                                                                                               34:66 )" #select
                                                                                                                  1:33 )" #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_CA_residues = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                  = universe_object.selectAtoms
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #find the center of geometry for each helix:
                                                                                                                                                                                                                                                                                                                                                                                                                          #select all the CA particles in each helix:
                                                                                                                                                                                                                                                                                                                                                                                                 in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                    and resid
                                                                                                                  and resid
                                                                                                                                                                                                                                                                                 resid
                                                                                                                                                                resid
                                                                                                                                                                                                                                                                                                                                                                           ref helix (y) | helix_2 (x) | helix_2 (y)\n')
                                                                                                                                                                 and
                                                                                                                                                                                                                                                                                  and
                                                                                                                                                                                                                                                                                                                              helix_tracking_file = open(output_file,'w')
                                                                                                                  CA
                                                                                                                                                                                                                                                                                 CA
                                                                                                                                                                CA
                                                                                                                                                                                                                                    name CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_2_CA_selection)
                                                                                                                      name
                                                                                                                                                                  name
                                                                                                                                                                                                                                                                                  name
  parse
                                                                                                                                                                                                                                                                                                                                                                                                                                               helix_1_CA_residues
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    be the reference
                        xtc files
                                                                                                                                                                                                                                   _
=
                                                                                                                                                               _
=
                                                                                                                  _
=
                                                                                                                                                                                                                                                                                  II
                                                                                                                      II
                                                                                                                                                                  II
                                                                                                                                                                                                                                       II
 trajectories or 'FGFR3' to
                                                                                                                   helix_1_CA_selection
                                                                                                                                                               helix_2_CA_selection
                                                                                                                                                                                                                                    helix_1_CA_selection
                                                                                                                                                                                                                                                                                 helix_2_CA_selection
                                                                                                                                                                                                            system_name == 'GpA':
                                                                                          system_name == 'FGFR3':
                       performed on
                                                                    box to the other).'''
                                                                                                                                                                                      FGFR3 monomer
                                                                                                                                             monomer
                                                                                                                                                                                                                                                                                                            monomer
                                               because of the
                                                                                                                                                                                                                                                              monomer
                        рe
                        should
                                                                                                                                                                                                             elif
                                                                                             if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            511
                                                                                                 495
                                                                                                                                                                      497
                                                                                                                                                                                                                                          499
                                                                                                                                                                                                                                                                                                                                \frac{5}{3}82
                                                                                                                                                                                                                                                                                                                                                                                                          503
                                                                                                                                                                                                                                                                                                                                                                                                                                                      505
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              509
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 507
```

```
splot' style. **Important: use with trajectories processed with trjconv -pbc
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_tracking_file.write(str(helix_1_plot_coordinate[0]) + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  always be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      10507. Will probably bin the data in a format appropriate for the gnuplot
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   thermal' plot similar to that used for Fig. 5 in Biochemistry (2008), 47,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    produce a
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 0,0 so might be able to stop writing that after testing)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          '''Similar to the 'relative_helical_motion' function but designed so that
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      dimer_symlink_directory, create_universe_selections, system_name, output_file):
                                                                                                                                                                                                                                                helix_1_center
                                                                                                                                                                                                                                                                                                                                            - helix_1_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             reference helix always faces the same direction to allow study of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  a and b. Copied from rmsfit.py
                                                                              helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              coordinates to file (helix 1 should
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                preferred dimerization interface (if there is one). Idea is to
                                                                          each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       + str(helix_1_plot_coordinate[1]) + ' '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           + str(
                                                                                                                                                                                                                                                             ı
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          527 def fixed_helix_thermal(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          -- frame: ' +
                                                                              from
                                                                                                                                                                                                                                                                                                                                            = helix_2center
                                                                                                                                                                                                                                                helix_1_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    return numpy.sqrt(numpy.sum(numpy.power(a-b,2))/a.shape[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               + ( - u / + +
                                                                          coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_plot_coordinate[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_2_plot_coordinate[0])
                                                                                                                                                                                                                                                             II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     print str(folder_name) +
                                                                                       center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              '''Returns RMSD between two coordinate sets
                                                                                                                                                                                                                                                                                                                                            helix_2_plot_coordinate
                                                                                                                                                                                                                                                     helix_1_plot_coordinate
                                                                              its
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #write the x,y
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             distributed with MDAnalysis.""
                                                                                   #so, subtract
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 mol and NOT -pbc nojump'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_tracking_file.close()
                                                                                                                                                                          frame
frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      compute_rmsd(a,b):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               def
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 523
                                                                                                    513
                                                                                                                                                                                                                                                                                                                                                              515
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                519
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         \frac{1}{2} \frac{1}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              525
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    517
```

```
= ['wildtype_dimer_replicate_1']
= "( name CA and resid 1:33 )" #select first FGFR3
                                                                                                                                                                                                                                  #for the reference structure I'm currently planning to use the first frame (and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              GpA
                                                    directly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     "(name CA and resid 34:66)" #select second
                          configuration as the rmsd reference for mutant
                                                                                                                               helix
                                                                                                                                                                                                                                                             or GpA.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              first
                                                                                                                                                                                                                                                                                      either
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           list (of length 1) for the
FGFR3
                                                                                                                                                                                                                                                                                                              these systems the function needs to know which folder the reference
                                                the three FGFR3 conditions to be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               and resid 24:46 )" #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #select
                                                                                                                                                                                                                                                             simulation for either FGFR3
                                                                                                                                                                                                                                                                                    allow creation of an MDAnalyis Universe and atom selection from
                                                                                                                              N
N
 three
                                                                                                                            || ref. helix y || helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          "( name CA and resid 1:23 )"
 structure for all
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ['GpA_dimer_replicate_1']
                                                                                                                                                                                                                                                                                                                                        which residues to select:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       script:
                                                                         comparable by visual inspection of the figure
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           create the (universe object, folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     a function defined in the head
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               name CA
 #challenge: can I use the same reference
                                                                                                                                                                                                                                                             dimer
                                                                                                                              outfile.write('#Format: ref. helix x
                                                                                                                                                     \normalfont{n'}) #not really what gets printed
                                                 of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               each
                                                                                                                                                                                                                                                             first helix) of the first WT
                                                                                                   outfile = open(output_file, 'w')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 II
                                                                                                                                                                                                                                                                                                                                         trajectories reside in and
                                                                                                                                                                                                                                                                                                                                                                                           ref_folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                   helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ref_folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_CA_selection
                          conditions (i.e., use WT
                                               well)? Basically, I want
                                                                                                                                                                                                                                                                                                                                                                 system_name == 'FGFR3':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        system_name == 'GpA':
                                                                                                                                                                              list_helix_1_centers = []
                                                                                                                                                                                                          list_helix_2_centers = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                   monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       system using
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #nom#
                                                                                                                                                                                                                                                                                                                                                                    if
      529
                                                                                                                                                                                                                                                                                                                                                                     384
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```

```
(0,0,0). We
                                                                                                                                                                                            reference_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               current_helix_1_CA_selection.coordinates
                                                                                                                                                                                                                                                                                                                                                                doing work
                                                                                                                                             = ref_universe_object.selectAtoms(helix_1_CA_selection)
                                                                       incorrectly
                                                                                                                                                                                                                                                                                                                                                                                                                                       each
                                                                                                                                                                                                                                                                                                                                                                                                                                                              going
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 frame. To keep things consistent in relative terms, perform
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          amount)
                                                                                                                                                                                                                                                                                                                                                                                                                                      in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #Find the (center of geometry zero'd) coordinates for helix 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                               are
                                                                                                                                                                                                                                                                                                                                         at
                                                                                                                                                                                                                                                                                                                                                                trajectory and
create_universe_selections(dimer_symlink_directory,
                                                                                                                                                                                                                                                                                                                                                                                                                                      again
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            universe_object.selectAtoms
                                                                                                                                                                                                                                                                                                                                        geometry
                                                                       had
                                                                                                                                                                                                                                                                                                                                                                                                                                                                We
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        2 (i.e., translate it by the same
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      current_helix_1_CA_selection.centerOfGeometry()
                                                                                                                       structure:
                                                                        you
                                                                                                                                                                                                                                                                                                                                                                                                                                                              because
                                                                                                                                                                                                                                                                                                                                                                                                                                      #Because helix 1 may move a bit we need to select it
                                                                                                                                                                geometry zero'd) coordinates:
                                                                    = universe_data[0][0] #careful here!
                                                                                                                                                                                                                                                                                                                                         Jo
                                                                                                                                                                                           ref_coordinates = reference_CA_selection.coordinates()
                                                                                                                                                                                                                                           for input to rms/transformation function:
                                                                                                                                                                                                                                                                                                                                                                                                                                                           N
                                               element in the sublist:
                                                                                                                                                                                                                                                                                                                                         center
                                                                                                                                                                                                                                                                                                                                                                in the
                                                                                                                                                                                                                                                                                                                                                                                                             for ts in universe_object.trajectory[::skip_frames]:
                                                                                                                     corresponding to the reference
                                                                                                                                                                                                                                                                                                                                                                                                                                                             select helix
                                                                                             reassigned universe_object which is very bad
                                                                                                                                                                                                                                                                                                                                        structure with
                                                                                                                                                                                                                                                                                                                                                                frames
                                                                                                                                                                                                                                                                 reference_CA_selection.masses()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             II
                                                                                                                                                                                                                                                                                                                                                                through
                                                                                                                                                                                                                                                                                                                                                                                                                                                              to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    track its relative position:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                current_helix_1_coordinates =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            current_helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         current_helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                              also want
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                        the reference
                                                                                                                                                                                                                                                                                                                                                               start looping
                                               first
                                                                                                                                                                    #get the reference (center of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        operation on helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                           frame. We will
                                              object is the
                        ref_folder_name_list)
                                                                                                                                           reference_CA_selection
                                                                                                                                                                                                                  .centerOfGeometry()
                                                                                                                     particles
                                                                       ref_universe_object
                                                                                                                                                                                                                                                                                                                                        #So, we now have
                                                                                                                                                                                                                                                                                                                                                                 ready to
   II
 universe_data
                                                                                                                     #select CA
                                               #universe
                                                                                                                                                                                                                                                                     II
                                                                                                                                                                                                                                                                                                                                                                                         there
                                                                                                                                                                                                                                           #need
    545
                                                                            547
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 559
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                                                                                                                                                                                                                                                                                                                       555
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               561
                                                                                                                                                                                                 551
                                                                                                                                                                                                                                                                                                                                          385
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<pre>current_helix_2_coordinates = current_helix_2_CA_selection.coordinates () - current_helix_1_CA_selection.centerOfGeometry() #We need to figure out what kind of transformation is needed for the best (rmsd) fit of current helix 1 back to the reference structure. This is based on the MDAnalysis/examples/rmsfit.py example code distributed with the most recent release of MDAnalysis: transformation = numpy.matrix(MDAnalysis.core.rms_fitting. rms_rotation_matrix(current_helix_1_coordinates,ref_coordinates, masses))</pre>	<pre>#Apply the transformation that puts helix 1 in the best (rmsd fit) match to the reference: helix_1_best_fit_coordinates = current_helix_1_coordinates * transformation #We want to perform the same transformation on helix 2 so that we maintain consistency relative to the reference structure in each frame (both helices must always experience the same transformation): helix_2_updated_coordinates = current_helix_2_coordinates * transformation</pre>	<pre>#After the transformations, both helices are represented as numpy matrices of coordinates. Since the CA particles all have the same mass, we can treat the mass as 1 and simply find the average position of all particles to represent the center of mass for each helix:</pre>
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```
center_of_mass_helix_1 = [x_sum/len(helix_1_best_fit_coordinates),y_sum
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #for x and y coordinate bin ranges we'll want to generated the bounds using
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       center
                                                                                                                                                                              x_sum += x; y_sum += y; z_sum += z
center_of_mass_helix_2 = [x_sum/len(helix_2_updated_coordinates)
                                                                                                                                                                                                                                                                                                 code
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          simulation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  geometric
                                                                                                                                                                                                                                                                                               accessed by the
                                                                                                                                                                                                                                                                                                                                                                                                                 str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        coordinates in the helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    = [x for (x, y, z) in list_helix_2_centers]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             list_helix_2_centers]
                                                                                                                                                                                                                                                                                                                                                       list_helix_1_centers.append(center_of_mass_helix_1)
                                                                                                                                                                                                                                                                                                                                                                                   list_helix_2_centers.append(center_of_mass_helix_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #now the function has finished looping through the current
                                                                                                                                                                                                                                                                                                                          will bin the data after the frame-stepping loop
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  because its
                                                                                                                                               for x, y, z in helix_2_updated_coordinates.tolist():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     mass for each helix
                                /len(helix_1_best_fit_coordinates),z_sum/len(
                                                                                                                                                                                                                                      len(helix_2_updated_coordinates),z_sum/len(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   based on the bounds of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                and
                                                                                                                                                                                                                                                                                               can be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            a bin separation and build bins around min
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #helix 1 is more or less fixed near the origin
                                                                                                                                                                                                                                                                                                                                                                                                                  -- frame:
                                                                                                                                                                                                                                                                                               centers in a list so they
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for (x,y,z) in
                                                      helix_1_best_fit_coordinates)]
                                                                                                                                                                                                                                                                helix_2_updated_coordinates)]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     min() and max() values for x and y
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   grabbing the appropriate centers of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 purposes
                                                                                                                    x\_sum=0; y\_sum=0; z\_sum=0
                                                                                                                                                                                                                                                                                                                                                                                                                 print str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            min(helix_2_x_coord_list)
                                                                                         #repeat for helix 2:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = [y
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for referencing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   bin value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_2_x_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_y_coord_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     list_of_bin_counts =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        bin_separation = 0.4
                                                                                                                                                                                                                                                                                                 #put the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           x_bin_value_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #populate the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                zeroed
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #set
                                                                                                                                                                                                                                                                                                                                                                                                                  387
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  599
                                                                                               579
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                                                                                                                                                       581
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  589
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```

```
appropriate x-bin for the x coordinate and we need to iterate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 list_of_bin_counts.append((x_bin,y_bin)
                                                                                                                                                                                                                                                                                                                                                                                                            ||
|
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    print
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            if helix_2_y > (y_bin - (bin_separation/2.0))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          and helix_2_y <= (y_bin + (bin_separation))
                                                                                                                                                                                                                                                                                                                                                                                                       if helix_2_x > (x_bin - (bin_separation/2.0)) and helix_2_x
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #if the above condition is satisfied then we have found the
                                                                                                                                                                                                                                                                                                             every x coordinate of helix 2 find a matching x-bin where it belongs:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                y coordinate fits:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     the x,y bin values in a tuple
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      coordinate falls in a 2D bin,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #every time the helix 2(x,y)
                                                                                                                                                                                                                                                                                                                                          for helix_2_x, helix_2_y, helix_2_z in list_helix_2_centers:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  append to a list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  through the y bins to see where the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for y_bin in y_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                       (x_bin + (bin_separation/2.0)):
                                                                                                                                                                                                                                                                                                                                                                        for x_bin in x_bin_value_list:
<= max(helix_2_x_coord_list):</pre>
                                                                                                                                                                                 while y <= max(helix_2_y_coord_list):
                                                                                                                                                                                                                y_bin_value_list.append(y)
                             x\_bin\_value\_list.append(x)
                                                                                                                       y = min(helix_2_y_coord_list)
                                                             x += bin_separation
                                                                                                                                                                                                                                                 y += bin_separation
                                                                                                                                                      y_bin_value_list = []
while x
                                                                                                                                                                                                                                                                                                                #for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      619
                                       601
                                                                                                                                                                                                                                                                                                                                                  611
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       615
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               617
                                                                                                  603
                                                                                                                                                              605
                                                                                                                                                                                                                         209
                                                                                                                                                                                                                                                                                       609
                                                                                                                                                                                                                                                                                                                                                                                                               613
                                                                                                                                                                                                                                                                                                                                                                                                                                        388
```

```
dimer_symlink_directory, create_universe_selections, heterodimer_helix_2_x_coord_list
      to
                                                                                                                                  outfile.write(str(x_bin) + ' ' + str(y_bin) + ' ' + str(float(
                                                                                                                                                                                                                                          gnuplot:
                                                                                                                                                                                                                                                                                                                                                                         each x bin value, iterate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for
                                                                                                                                                                                                                                                                                                                                                                                                            column)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    the
 sorted by default so it should be sensible
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  consider the data from all 10 FGFR3 replicates
                                                                                                                                                                                                                                                                                                                                          z data with the same
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Jo
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      some extraneous things in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                each of the three simulation conditions and produce data intended for
                                                                                                                                                                  list_of_bin_counts.count((x_bin,y_bin)))/float(len(
list_helix_2_centers))) + '\n')
                                                                                                                                                                                                                                      an empty line between the blocks of (x-bin) data for
                                     each
                                                                                                                                                                                                                                                                                                                                                                                                           z value in third
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix in the first frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              similarly named function in this module.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               def fixed_helix_thermal_merged(folder_name, universe_object, skip_frames,
                                   gnuplot-ready probability for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               either."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             , heterodimer_helix_2_y_coord_list, mutant_helix_2_x_coord_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              \verb|mutant_helix_2_y_coord_list|, wildtype_helix_2_x_coord_list|,
                                                                                                                                                                                                                                                                                                                                         #gnuplot splot wants space-separated blocks of x, y,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     ['wildtype_dimer_replicate_1']
                                                                                                                                                                                                                                                                                                                                                                         number of points in each block (i.e., a block for
                                                                                                                                                                                                                                                                                                                                                                                                           giving the corresponding
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  code that weren't really needed in the original
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                summary plot. I will probably remove
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   simply the first
                                                                                                     for y_bin in y_bin_value_list:
                                          ದ
#the x and y bin lists should be
                                   loop through each and produce
                                                                     for x_bin in x_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        structure is
                                                                                                                                                                                                                                                                                                                                                                                                           through all y bin values
                                                                                                                                                                                                                                                                      outfile.write('\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ''Modified version of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                function is designed to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                wildtype_helix_2_y_coord_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ref_folder_name_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   list_helix_2_centers =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     simulation
                                                                                                                                                                                                                                           #we want
                                                                                                                                                                                                                                                                                                        outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #the reference
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     first WT
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      single
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                \frac{8}{389}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               635
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             633
                                                                              621
                                                                                                                                              623
                                                                                                                                                                                                                                                                                 625
                                                                                                                                                                                                                                                                                                                                                 627
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        631
```

```
first FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                            (0,0,0). We
                                                                                                                                                                                                                                                                                                    ref\_coordinates = reference\_CA\_selection.coordinates() - reference\_CA\_selection
                                                                                                                                                                                                                                                        reference_CA_selection = ref_universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   doing work
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               t
0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              going
                                                                                            the
                       34:66 )" #select second FGFR3
                                                                                            for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               are
                                                                                                                                                                                                                                                                                                                                                                                                                                           of geometry at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   trajectory and
                                                                                                                                        create_universe_selections(dimer_symlink_directory,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         current_helix_1_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     again
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    current_helix_2_CA_selection = universe_object.selectAtoms
                                                                                          1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                M
M
                                                                                       folder_name) list (of length
                                                                                                                                                                                                                                   corresponding to the reference structure:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          2 because
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     it
1:33 )" #select
                                                                                                                                                                                                                                                                             geometry zero'd) coordinates:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      a bit we need to select
                                                                                                                                                                                                                                                                                                                                                  masses for input to rms/transformation function:
                                                                                                                  script:
                                                                                                                                                                                    sublist:
                                                                                                                                                                                                                                                                                                                                                                                                                                            structure with center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for ts in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           frame. We will also want to select helix
                                                                                                                 a function defined in the head
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 frames
                                                                                                                                                                                     #universe object is the first element in the
 resid
                       resid
                                                                                                                                                                                                         = universe_data[0][0]
                                                                                                                                                                                                                                                                                                                                                                         reference_CA_selection.masses()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 its relative position:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 through
                       and
   and
                                                                                         (universe object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #Because helix 1 may move
 CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                            the reference
"( name
                      " ( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                start looping
                                                                                                                                                                                                                                                                               #get the reference (center of
                                                                                                                                                              ref_folder_name_list)
                       П
    II
                                                                                                                                                                                                                                                                                                                             .centerOfGeometry()
                                                                                                                                                                                                                                  particles
 helix_1_CA_selection
                        helix_2_CA_selection
                                                                                                                                                                                                           ref_universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                                            #So, we now have
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   track
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ready to
                                                                                            #now create the
                                                                                                                    system using
                                                                                                                                         universe_data
                                                                                                                                                                                                                                  CA
                                                                                                                                                                                                                                                                                                                                                                          II
                                                                                                                                                                                                                                  #select
                                                                                                                                                                                                                                                                                                                             390 25
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            655
                              637
                                                                                                                                                                                                                                       643
                                                                                                639
                                                                                                                                                                                                                                                                                    645
                                                                                                                                                                                                                                                                                                                                                                                                     649
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           653
                                                                                                                                                                                          641
                                                                                                                                                                                                                                                                                                                                                                                                                                                 651
```

helix_2_CA_selection

```
x_sum += x; y_sum += y; z_sum += z
center_of_mass_helix_2 = [x_sum/len(helix_2_updated_coordinates),y_sum/
                                                                                                                                           current_helix_1_coordinates = current_helix_1_CA_selection.coordinates
                                                                                                                                                                                                                                           current\_helix_2\_coordinates = current\_helix_2\_CA\_selection.coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                        best (rmsd) fit of current helix 1 back to the reference structure.
    each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                all particles to represent the
                                                                                                                                                                                                                                                                                                                                                                                         #We need to figure out what kind of transformation is needed for the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   \#We don't actually need to apply the transformation to helix 1--\mathrm{just}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #Helix 2 is represented as a numpy matrix of coordinates. Since the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              code
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       rms_rotation_matrix(current_helix_1_coordinates,ref_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               particles all have the same mass, we can treat the mass as 1
                                                                                             amount)
                                                   terms, perform
  coordinates for helix 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              example
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 want to know where helix 2 is relative to 1 now that 1 is
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  distributed with the most recent release of MDAnalysis:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      = numpy.matrix(MDAnalysis.core.rms_fitting
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_2_updated_coordinates = current_helix_2_coordinates
                                                                                          operation on helix 2 (i.e., translate it by the same
                                                                                                                                                                                            current_helix_1_CA_selection.centerOfGeometry()
                                                                                                                                                                                                                                                                                         current_helix_1_CA_selection.centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      is based on the MDAnalysis/examples/rmsfit.py
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for x, y, z in helix_2_updated_coordinates.tolist():
                                                 frame. To keep things consistent in relative
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       len(helix_2_updated_coordinates),z_sum/len(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              average position of
geometry zero'd)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             x_sum=0; y_sum=0; z_sum=0
#Find the (center of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           simply find the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            center of mass.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      transformation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      transformation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   masses))
```

629

661

299

665

663

391

```
contain the approrpiate set of coordinates from 10 simulations. For binning
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          the portion
                                                                                                                                                                                                                                  00
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        after this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    the lists
                             code
                                                                                                                                                                     they
                                                                                                                                                                                                filtered by simulation type (i.e., folder name) and append them to
                                                                                                                                                                                                                           initialized in the head
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         generalize
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     a function and then call it
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       thermal_bins(helix_2_x_coord_list, helix_2_y_coord_list, output_file):
                             the
                                                                                                                                                                     that
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    οĮ
                                                                                                               str(ts.frame)
                            accessed by
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    each
                                                                                                                                                                     2 such
                                                     will bin the data after the frame-stepping loop list_helix_2_centers.append(center_of_mass_helix_2)
                                                                                                                                                                                                                                                                                                                                                                    heterodimer_helix_2y_-coord_-list.append_{
m (y)}
                                                                                                                                                                                                                                                                                                                                       \mathtt{heterodimer\_helix\_2\_x\_coord\_list.append(x)}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 simulations,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         and probability calculation it will be worthwhile to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 wildtype_helix_2_x_coord_list.append(x)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          "ildtype\_helix\_2\_y\_coord\_list.append(y)
                                                                                                                                                                     extract the x and y coordinates for helix
                                                                                                                                                                                                                                                       they can be appended as the function loops through)
                                                                                                                                                                                                                                                                                                                                                                                                                                                   mutant_helix_2x_coord_list.append(x)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                \mathtt{mutant\_helix\_2\_y\_coord\_list.append(y)}
                                                                                                            ' -- frame: ' +
                             рe
                                can
                                                                                                                                                                                                                         appropriately named list (these lists are
                                                                                                                                                                                                                                                                                                        for (x,y,z) in list_helix_2_centers:
                                                                                                                                                                                                                                                                                                                                                                                                                      for (x,y,z) in list_helix_2_centers:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      in list_helix_2_centers:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 going through all 30 FGFR3 dimer
                             list so they
helix_2_updated_coordinates)]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     deals with that in
                                                                                                                  +
                                                                                                                                                                                                                                                                                  'heterodimer' in folder_name:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  script
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'wildtype' in folder_name:
                                                                                                               str(folder_name)
                                                                                                                                                                                                                                                                                                                                                                                             'mutant' in folder_name:
                                centers in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                head
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 function from the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      of the code that
                                                                                                               print
                                                                                                                                                                      want to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    after
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #so,
                                                                                                                                                                     #Me
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       def
                                                                                                                                                                                                                                                                                                                                                                           679
                                                                                                                                                                                                                                                                                                                                                                                                392^{\frac{7}{89}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          689
                                    671
                                                                                                                     673
                                                                                                                                                                          675
                                                                                                                                                                                                                                                                                                                  677
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             685
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   687
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       683
```

```
binning
                          probability data
                                                                                                                                        Note
                                                    appropriate file. Meant to work with the *overall* FGFR3 'thermal'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  every x coordinate of helix 2 find a matching x-bin where it belongs:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_x,helix_2_y in zip(helix_2_x_coord_list,helix_2_y_coord_list):
                                                                                position data, so the lists contain coordinates from all 10 replicates
                                                                                                                                          script.
                                                                                                                                                                   that it contains its own
                                                                                                            conditions. Call this function after
                                                                                                                                     main) in the head
                            binned
                                                                                                                                                                                                                                                                                separation and build bins around min and max values
 data from the
                                                                                                                                                                                                                                                                                                                                                                   data
                          fixed_helix_thermal_merged() function and writes the
                                                                                                                                                                                                                                                                                                                                                                 of
                                                                                                                                                                                                                                                                                                                                                                 the bounds
                                                                                                                                                                    in
                                                                                                                                        of
                                                                                                                                                                                          you don't have to call this.'''
                                                                                                                                       (and outside
                                                                                                                                                                the non-merged function is different
'''Parses the FGFR3 helix 2 coordinate list
                                                                                                                                                                                                                                                                                                                                                                      On
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       for x_bin in x_bin_value_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                  <= max(helix_2_x_coord_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     while y <= max(helix_2_y_coord_list)
                                                                                                                                                                                                                                                                                                                                                                 based
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              	exttt{x\_bin\_value\_list.append}(	exttt{x})
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                y\_bin\_value\_list.append(y)
                                                                                                                                                                                                                                                   outfile = open(output_file, 'w')
                                                                                                            each of the three simulation
                                                                                                                                       fixed_helix_thermal_merged()
                                                                                                                                                                                                                                                                                                                                                                #populate the bin value lists
                                                                                                                                                                                                                                                                                                                                                                                           x = min(helix_2_x_coord_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = min(helix_2_y_coord_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              y += bin_separation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         x += bin_separation
                                                                                                                                                                                                                                                                                                                                                                                                                      x_bin_value_list = []
                                                                                                                                                                                                                                                                                                                                       list_of_bin_counts =
                                                                                                                                                                                                                                                                                                         bin_separation = 0.4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        y_bin_value_list
                                                                                                                                                                                               routines so
                                                                                                                                                                                                                                                                                a bin
                                                     to the
                                                                                                                                                                                                                                                                                                                                                                                                                                                      ×
                                                                                                                                                                                                                                                                                #set
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #for
                                                                                                                                                                                                                                                                                                                                                                                              §
393
                                                                                                                                                                                                                                693
                                                                                                                                                                                                                                                                                       695
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     705
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  709
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       711
                                                                                                                                                                                                                                                                                                                                            697
                                                                                                                                                                                                                                                                                                                                                                                                                                                          701
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                703
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           707
```

```
appropriate x-bin for the x coordinate and we need to iterate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            list_of_bin_counts.append((x_bin,y_bin)
if helix_2_x > (x_bin - (bin_separation/2.0)) and helix_2_x <=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #the x and y bin lists should be sorted by default so it should be sensible to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               outfile.write(str(x_bin) + ' ' + str(y_bin) + ' ' + str(float(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  \#we want an empty line between the blocks of (x-bin) data for gnuplot:
                                                                                                                                                                                                                                                                                                                                                                                                                                                     print
                                                                                                                                                                                                                                                                                     if helix_2_y > (y_bin - (bin_separation/2.0))
                                                                                                                                                                                                                                                                                                                            and helix_2_y <= (y_bin + (bin_separation))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        a gnuplot-ready probability for each 2d bin
                                                                              #if the above condition is satisfied then we have found the
                                                                                                                                                                                                         for y_bin in y_bin_value_list: #the x,y coordinates
                                                                                                                                                                  through the y bins to see where the y coordinate fits:
                                                                                                                                                                                                                                                                                                                                                                                                                                                     coordinate falls in a 2D bin,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            the x,y bin values in a tuple
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                list_of_bin_counts.count((x_bin,y_bin)))/float(len(
helix_2_x_coord_list))) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                            #every time the helix 2 (x,y)
                                                                                                                                                                                                                                                should match because lists are ordered
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   append to a list:
                                         (x_bin + (bin_separation/2.0)):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               for y_bin in y_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            loop through each and produce
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for x_bin in x_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           outfile.write('\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ີ້ເ
394
                                                                                            715
                                                                                                                                                                                                                                                                                               717
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             729
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  723
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 727
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                725
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             721
```

<pre>def track_bilayer_thickness(folder_name, universe_object, skip_frames, dimer_symlink_directory, outfile):</pre>	(FGFR3 or GpA) won't matter and both will have output files with the same name	the top level; basically it has dependencies that some users might not have but I want to use this module to simplify the leaflet selection process outfile = open(outfile,'w')	e.write('#column format	rse_o aflet	<pre>leaflets = MDAnalysis.analysis.leaflet.LeafletFinder(universe_object, ' resname POP* and name P*')</pre>	<pre>leaflet_1_phosphate_selection = leaflets.atoms(0) #atom selection object for first leaflet</pre>
187	30	95	739	741	743	745

```
absolute value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Z coordinate motion for helix 1 relative to helix 2 (i.e., since
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_1_CA_selection = "( name CA and resid 1:33 )" #select first FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              the bilayer (defined as the average of the center of mass of the two leaflet
                                                                                                                                                                                                                                    bilayer\_thickness = abs(leaflet\_1\_phosphate\_selection.centerOfGeometry
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             frame interval. This function should only be used on trajectories that have
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       system_name' flag) to a specified gnuplot-ready output file for a specified
                                                                                                                        for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ''Prints the Z coordinate of the geometric center *relative to the center of
                                                                                                                                                                                                                                                                                                                                                     ' + str(bilayer_thickness) + '\langle n \rangle'
                                                                                                                      geometry
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helices (based on
selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            def geo_Z_tracking_relative_to_bilayer(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    centering will reduce the
                                                                                                                                                                                                                                                                        ()[2] - leaflet_2_phosphate_selection.centerOfGeometry()[2])
                                                                                                                                                                                               of this difference is an estimate of bilayer thickness
                                                                                                                                                         the phosphate populations in each of the bilayers. The
                                                                                                                    coordinates of the respective centers of
 #atom
                                                                                                                                                                                                                                                                                                                                                                                                                                    str(ts.frame)
 leaflets.atoms(1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                phosphate populations)* for each of the GpA or FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                                  ' -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                not been centered by GROMACS trjconv because
       II
                                                                                                                                                                                                                                                                                                                                                       outfile.write(str(ts.frame) +
   leaflet_2_phosphate_selection
                                                                                                                                                                                                                                                                                                                                                                                                #track progress of iteration:
                                           second leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       import MDAnalysis.analysis.leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                print str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           always center helix 1).'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            if system_name == 'FGFR3':
                                                                                                                    #Subtract the Z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               system_name, output_file):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            amplitude of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        396
                                                                                        747
                                                                                                                                                                                                                                                                                                                                                                                                                                            753
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         755
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 759
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              761
                                                                                                                                                                                                                                              749
                                                                                                                                                                                                                                                                                                                                                                 751
```

```
= universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                                                               = universe_object.selectAtoms(helix_2_CA_selection)
                                                                                                                                                                        coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   {\tt middle\_Z\_position\_of\_bilayer} = (leaflet\_1\_selection.centerOfGeometry()
                                                                GpA
                                                                                                                                                                                                                                                                                                                        = MDAnalysis.analysis.leaflet.LeafletFinder(universe_object,
                                                                                                                                                                                                                                                                                                                                             better with phosphate only for
                                                                                                         second
   second
                                                                                                                                                                                                                                                                                                                                                                                                                                for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          for
                                                               first
                                                                                                                                                                                                                                                                                                                                                                                       something
                                                                                                                                                                                                                                                                                                                                                                   the full POPC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            geometry of each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          object
                                                                                                                                                                                                                                                                                                                                                                                                                                 object
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        geometric
34:66 )" #select
                                                                                                        #select
                                                                                                                                                                      Z_outfile.write('#column 1: frame # || column 2: helix 1 relative Z
                                                                #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      [2] + leaflet_2_selection.centerOfGeometry()[2]) / 2.0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  take the result to be an estimate of the bilayer
                                                                                                                                                                                                                                                                                                                                                                                     molecules by reducing the cutoff from 15 Angstrom to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          selection
                                                                                                                                                                                                                                                                                                                                                                                                                                 selection
                                                                                                        24:46 )"
                                                              =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       positions of the helical
                                                                                                                                                                                                                                                                                                                                                                  now; but you could probably get it to work with
                                                               1:23
                                                                                                                                                                                                                                                                                                                                                                                                                                #atom
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #atom
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              of the center of
                                                                                                                                                                                                                in universe_object.trajectory[::skip_frames]:
  and resid
                                                               and resid
                                                                                                        resid
                                                                                                                                                                                           || column 3: helix 2 relative Z coordinate\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         leaflets.atoms(1)
                                                                                                                                                                                                                                                                                                                                                                                                                                leaflets.atoms(0)
                                                                                                                                                                                                                                                                                                                                            POP* and name P*') #behaves
                                                                                                         and
 CA
                                                               CA
                                                                                                        CA
    name
                                                                 name
                                                                                                          name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Z coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            a relative position
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Ζ
                                                                                                                                                 = open(output_file, 'w')
                                                                                                        _
=
                                                                                                                                                                                                                                                         centerOfGeometry()[2]
                                                                                                                                                                                                                                                                                                 centerOfGeometry()[2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        subtract the
    II
                                                                                                         II
                                                                  II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             II
                                                                                                                                                                                                                                                                                                                                                                                                                                    II
 helix_2_CA_selection
                                                              helix_1_CA_selection
                                                                                                         helix_2_CA_selection
                                                                                                                                                                                                                                   helix_1_CA_center_Z
                                                                                                                                                                                                                                                                               helix_2_CA_center_Z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          leaflet_2_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                 leaflet_1_selection
                                       == 'GpA':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                second leaflet
                      FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                      first leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #We average the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       which we
                                                                                                                                                                                                                                                                                                                                                resname
                                                                                       monomer
                                                                                                                                                                                                                                                                                                                          leaflets
                                            system_name
                                                                                                                                                  Z_outfile
                                                                                                                                                                                                                  for ts
                                                763
                                                                                                                                                                                                                                          692
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               773
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   775
                                                                                                              765
                                                                                                                                                                            767
                                                                                                                                                                                                                                                                                                     397^{\frac{7}{5}}
```

```
"( name CA and resid 1:33 )" #select first FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     second GpA
                                                                                                                                   ' + str(relative_Zcoordinate_helix_1)
                                                                                                                                                                                                                                                                                                                          '''Should track the (interphosphate) thickness of the bilayer in the immediate
                                                                                                                                                                                                                                                                                                                                                                                 bilayer thickness spikes down really low for certain simulations. Algorithm
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  outfile.write('#column 1: frame # || column 2: local leaflet interphosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              34:66 )" #select second
                                                                                                                                                                                                                                                                           system_name
                                                                                                                                                                                                                                                                                                                                                        because
                                                                                                         #print relative_Zcoordinate_helix_1, relative_Zcoordinate_helix_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  24:46 )" #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             1:23 )" #select
 = middle_Z_position_of_bilayer
                                                   middle_Z_position_of_bilayer
                                                                                                                                                                                                                                                                                                                                                       vicinty of the peptide dimer. Still buggy as of August 2/ 2010
                                                                                                                                                                                         str(ts.frame)
                                                                                                                                                                                                                                                                         skip_frames,
                                                                                                                                                             ' + str(relative_Zcoordinate_helix_2) + ' n'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            (A) \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       separation (Angstroms) || column 3: global Z sep.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               and resid
                                                                                                                                                                                       -- frame: ' +
                                                                                                                                                                                                                                                                         def local_bilayer_thickness(folder_name, universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    CA
                                                                                                                                     Z_outfile.write(str(ts.frame) +
                                                        II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  = "( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              " ( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 name
relative_Zcoordinate_helix_1
                                                  relative_Zcoordinate_helix_2
                                                                                                                                                                                                                                                                                                                                                                                                                                      import MDAnalysis.analysis.leaflet
                                                                                                                                                                                       print str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      = open(output_file,'^{\text{w'}})
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 II
                                                                                helix_2_CA_center_Z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                if system_name == 'FGFR3':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   system_name == 'GpA':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            monomer
                                                                                                                                                                                                                   Z_outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                        problem?'''
                                                                                                                                                                                                                                                                                                 output_file):
                                                                                                                                                                                                                                                                              783
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        793
                                                            777
                                                                                                                                           779
                                                                                                                                                                                                                          781
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          789
                                                                                                                                                                                                                                                                                                                                                                                                                                              785
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  787
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         791
                                                                                                                                                                                                                                                                                                                                                                                     398
```

```
of the leaflets, but limit to selecting atoms with x or y coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    local_selection_string = '( ( prop x <= %s and prop x >= %s ) or ( prop
y <= %s and prop y >= %s ) ) and resname POP* and name P*' % (7.0 +
average_helical_center_X, average_helical_center_X - 7.0, 7.0 +
average_helical_center_Y, average_helical_center_Y - 7.0)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               leaflet_2_selection = local_leaflet_phosphates.atoms(1) #atom selection
                                                                                                                                        universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                      helix_2_CA_center_X = universe_object.selectAtoms(helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                              helix_1_CA_center_Y = universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_CA_center_Y = universe_object.selectAtoms(helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                within 7 Angstroms of the average x or y coordinates of the helical
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #use MDAnalysis leaflet selection feature to pick phosphates from each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       average_helical_center_X = (helix_1_CA_center_X + helix_2_CA_center_X)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       = (helix_1_CA_center_Y + helix_2_CA_center_Y)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      local_leaflet_phosphates = MDAnalysis.analysis.leaflet.LeafletFinder(
                                                    centers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               = local_leaflet_phosphates.atoms(0) #atom
                                                                                      two helices (assumes TM peptides and bilayer in xy plane):
                                             geometric
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             geometric centers (i.e., local phosphates):
                                               average of the x coordinates of the
in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    universe_object, local_selection_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               object for second leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               object for first leaflet
                                                                                                                                                                                                                                                                            centerOfGeometry()[0]
                                                                                                                                                                                                                                                                                                                                                                            \tt centerOfGeometry()[1]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       \tt centerOfGeometry()[1]
                                                                                                                                                                                   centerOfGeometry()[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     average_helical_center_Y
                                                                                                                                        helix_1_CA_center_X =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    leaflet_1_selection
  for ts
                                                           795
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         803
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                802
                                                                                                                                                                                                                                                  797
                                                                                                                                                                                                                                                                                                                                                                                                                                          799
```

```
- global_leaflet_2_selection.centerOfGeometry()
   each leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  rmsd-fixed frame of the first helix to produce gnuplot-ready data with frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       This function
                                                                                                                                                                                          #for comparison print the global bilayer thickness in the third column
                                                                                                                                                                                                                                                             global_leaflet_phosphates = MDAnalysis.analysis.leaflet.LeafletFinder(
                                                                                                                                                  centerOfGeometry()[2] - leaflet_2_selection.centerOfGeometry()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             structures for the
                                                                                                                                                                                                                                                                                                                                                                                                                  {\tt global\_interphosphate\_Z\_separation} = {\tt abs(global\_leaflet\_1\_selection.}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          simulation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 seems to be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          geometry) position of the second helix in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            dimer_symlink_directory, create_universe_selections, system_name, output_file):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    819 def frame_abstracted_relative_position(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 to read in the
                                                                                                                                                                                                                                                                                                                                                                                 global_leaflet_phosphates.atoms(1)
                                                                                                                                                                                                                                                                                                                                           global_leaflet_phosphates.atoms(0)
                                                                                                              local_interphosphate_Z_separation = abs(leaflet_1_selection.
   centers of
                                        local
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         # on the Z-axis so you can follow the x and y positions as the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     trjconv flags).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         proceeds from bottom to top of 3D plot. Since the RMSD fixing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       str(ts.frame)
                                        the
                                                                                                                                                                                                                         (i.e., to see if the local differs from overall)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 okay with alpha carbon atom selections, should be able
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           is largely for the purpose of obtaining representative
                                         to estimate
geometric
                                                                                                                                                                                                                                                                                                       universe_object, 'resname POP* and name P*')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 global_interphosphate_Z_separation) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           local_interphosphate_Z_separation) + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     centered CA trajectories (-center and -pbc mol
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       outfile.write(str(ts.frame) + ' ' + str(
   Ν
                                        phosphate population can be used
 difference in
                                                                                                                                                                                                                                                                                                                                         global_leaflet_1_selection =
                                                                                                                                                                                                                                                                                                                                                                                global_leaflet_2_selection =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            x and y (center of
                                                                                                                                                                                                                                                                                                                                                                                                                                                        centerOfGeometry()[2]
   #the magnitude of the
                                                                            thickness:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         print
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ''Tracks the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      400
           807
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     817
                                                                                                                                                                                                  809
                                                                                                                                                                                                                                                                                                                                                     811
                                                                                                                                                                                                                                                                                                                                                                                                                             813
```

```
from other functions in this module. " "
                                                                                                                                                                                                                                                                                                                                                                                                     = ['wildtype_dimer_replicate_1']
= "( name CA and resid 1:33 )" #select first FGFR3
                                                                                                                                                                                                                                        #for the reference structure I'm currently planning to use the first frame (and
                                                                                                                                 position
                                                   a data (dimer conformation) point of
                         easier to track down the frame
 example, with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  "(name CA and resid 34:66)" #select second
                                                                                                                                                                                                                                                                    dimer simulation for either FGFR3 or GpA.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              first
                                                                                                                                                                                                                                                                                              either
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              list (of length 1) for the
                                                                                                                                                                                                                                                                                                                      these systems the function needs to know which folder the reference
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               and resid 24:46 )" #select
                                                                                                                                    5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #select
                                                                                                                                                                                                                                                                                           allow creation of an MDAnalyis Universe and atom selection from
                                                                                                                                 COM
on the thermal plots. For
                                                                                                                                 0
                                                                                                                                COM x position (A) || helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          "( name CA and resid 1:23 )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ['GpA_dimer_replicate_1']
                                                                                                                                                                                                                                                                                                                                                 trajectories reside in and which residues to select:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         script:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              create the (universe object, folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         a function defined in the head
                           should be much
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                name CA
                                                                                                                                                       ) || frame # (starts at 1 for MDA)\langle n^{-1} \rangle
                                                   axis of plot) corresponding to
                                                                             interest. Will definitely borrow code
different interaction faces based
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                first helix) of the first WT
                                                                                                                               outfile.write('#Format: helix 2
                                                                                                      outfile = open(output_file,'w')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 II
                           3D plots it
                                                                                                                                                                                                                                                                                                                                                                                                      ref_folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ref_folder_name_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                           system_name == 'FGFR3':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       system_name == 'GpA':
                                                                                                                                                                                                             list_helix_2_centers = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              FGFR3 monomer
                                                                                                                                                                                     list_helix_1_centers =
                                                                                                                                                                                                                                                                                                                                                                                                                                                               monomer
                         οĘ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              monomer
                            new set
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           system using
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #uom
                                                                                                                                                                                                                                                                                                                                                                              if
                                                                                                                                                                                                                                                                                                                                                                          401 $\frac{5}{20}$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     835
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       833
                                                                                                                                                                                             823
                                                                                                                                                                                                                                                825
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           829
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        831
                                                                                                              821
```

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(0,0,0). We
                                                                                                                                                                                                                                      ref_coordinates = reference_CA_selection.coordinates() - reference_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              current_helix_1_CA_selection.coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                        trajectory and doing work
                                                                                                                                                                              = ref_universe_object.selectAtoms(helix_1_CA_selection)
                                                                                       incorrectly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      frame. To keep things consistent in relative terms, perform the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             going
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   operation on helix 2 (i.e., translate it by the same amount)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #Because helix 1 may move a bit we need to select it again in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #Find the (center of geometry zero'd) coordinates for helix 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             we are
                                                                                                                                                                                                                                                                                                                                                                                        geometry at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     current_helix_1_CA_selection = universe_object.selectAtoms
create_universe_selections(dimer_symlink_directory
                                                                                       had
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              current_helix_1_CA_selection.centerOfGeometry()
                                                                                                                                                  structure:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             because
                                                                                         you
                                                                                                                                                                                                      geometry zero'd) coordinates:
                                                                                   = universe_data[0][0] #careful here!
                                                                                                                                                                                                                                                                                                                                                                                          O.f
                                                                                                                                                                                                                                                                                                    for input to rms/transformation function:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          select helix 2
                                                       element in the sublist:
                                                                                                                                                                                                                                                                                                                                                                                           center
                                                                                                                                                                                                                                                                                                                                                                                                                        in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ts in universe_object.trajectory[::skip_frames]:
                                                                                                                                                 corresponding to the reference
                                                                                                                   reassigned universe_object which is very bad
                                                                                                                                                                                                                                                                                                                                                                                          structure with
                                                                                                                                                                                                                                                                                                                                                                                                                        through frames
                                                                                                                                                                                                                                                                                                                               reference_CA_selection.masses()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        its relative position:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          frame. We will also want to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 current_helix_1_coordinates =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             current_helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                          #So, we now have the reference
                                                                                                                                                                                                                                                                                                                                                                                                                       start looping
                                                         first
                                                                                                                                                                                                          #get the reference (center of
                                                       object is the
                           ref_folder_name_list)
                                                                                                                                                                            reference_CA_selection
                                                                                                                                                                                                                                                                    .centerOfGeometry()
                                                                                                                                                 particles
                                                                                       ref_universe_object
                                                                                                                                                                                                                                                                                                                                                                                                                          ready to
   II
universe_data
                                                                                                                                               #select CA
                                                                                                                                                                                                                                                                                                                                  II
                                                                 837
                                                                                                                                                                                                                                                                                                          843
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            849
                                                                                                                                                       839
                                                                                                                                                                                                                                                                                                                                                                   845
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 851
                                                                                                                                                                                                                841
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         847
                                                                                                                                                                                                                                                                                                                                                                                                                          402
```

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position
                                                                                                                                                                                                                                                                                                                                                                                                                                                               transformation):
current_helix_2_CA_selection.coordinates
                                                                                                           structure
                                                                                 needed for the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 all have the same
                                                                                                                                                                                                                                                                                                                                                                                                                                    each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            in helix_1_best_fit_coordinates.tolist(): #convert numpy
                                                                                                                                       code
                                                                                                                                                                                                                  rms_rotation_matrix(current_helix_1_coordinates,ref_coordinates
                                                                                                                                                                                                                                                                                                 fit)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           the average
                                                                                                                                                                                                                                                                                                                                                                                                                                     maintain consistency relative to the reference structure in
                                                                                                                                                                                                                                                                                                                                                                                                            that
                                                                                                                                      example
                                                                                                                                                                                                                                                                                                  (rmsd
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #After the transformations, both helices are represented as
                                                                                                          current helix 1 back to the reference
                                                                                                                                                                                                                                                                                                                                                     current_helix_1_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                              0
                                                                                                                                                             distributed with the most recent release of MDAnalysis:
                                                                                                                                                                                       = numpy.matrix(MDAnalysis.core.rms_fitting
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         = current_helix_2_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      mass for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #calc com helix 1: [We can probalby ignore helix 1 when
                                                                                                                                                                                                                                                                                                 the best
                                                                                                                                                                                                                                                                                                                                                                                                                                                               always experience the same
                                                                                                                                                                                                                                                                                                                                                                                                          transformation on helix 2
                         current_helix_1_CA_selection.centerOfGeometry()
                                                                                 transformation is
                                                                                                                                 is based on the MDAnalysis/examples/rmsfit.py
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           find
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     of all particles to represent the center of
                                                                                                                                                                                                                                                                                                 in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1 and simply
                                                                                                                                                                                                                                                                                                  \leftarrow
                                                                                                                                                                                                                                                                                                puts helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      matrix object to list for std indexing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Since the CA
                                                                                #We need to figure out what kind of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            the mass as
                                                                                                                                                                                                                                                                                                  #Apply the transformation that
                                                                                                                                                                                                                                                                                                                                                         II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           things are working properly]
current_helix_2_coordinates =
                                                                                                                                                                                                                                                                                                                                                                                                              same
                                                                                                                                                                                                                                                                                                                                                       helix_1_best_fit_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_2_updated_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   coordinates.
                                                                                                                                                                                                                                                                                                                                                                                                                                                             frame (both helices must
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    x_sum=0; y_sum=0; z_sum=0
                                                                                                                                                                                                                                                                                                                            the reference
                                                                                                                                                                                                                                                                                                                                                                                                          perform the
                                                                                                          best (rmsd) fit of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              treat
                                                                                                                                                                                                                                                                                                                                                                                 transformation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     transformation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              can
                                                                                                                                                                                          transformation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 οĘ
                                                                                                                                                                                                                                                                                                                                                                                                          #We want to
                                                                                                                                                                                                                                                                                                                            match to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               mass, we
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     matrices
                                                                                                                                                                                                                                             masses))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               for x,y,z
                                                                                                                                   This
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855

857

859

403

methods

865

863

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(August 7/2010)'''
                             center_of_mass_helix_1 = [x_sum/len(helix_1_best_fit_coordinates),y_sum
                                                                                                                                                                                                                                                                                center_of_mass_helix_2 = [x_sum/len(helix_2_updated_coordinates),y_sum/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   per frame. Uses the fast algorithm built-in to MDAnalysis which is coded in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     closest contacts, but that would be a problem for retrieving data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           contacts data that doesn't skip frames so
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                def closest_contacts_efficient(folder_name, universe_object, skip_frames, system_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   script for CA-CA distances
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               outfile.write('#format: column 1: frame number || column 2: closest approach
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              along with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   every 10th
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        make
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               every 25th frame starting at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       sure helix 1 properly centered at 0,0 for plotting purposes
                                                                                                                                                                                                                                                                                                                                                                                                                          center_of_mass_helix_2[1]) + ' ' + str(ts.frame) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              can more easily use the resulting data files to plot a scatter helix crossing angle (for which I have every 25th frame startin
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               specific frame depending on simulation). Originally I only had
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    center_of_mass_helix_1[0],center_of_mass_helix_1[1]
                                                                                                                                                                                                                                                                                                                                                                                       outfile.write(str(center_of_mass_helix_2[0]) + ' ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                -- frame: ' + str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helix crossing.
                                                                                                                                                                                                             for x,y,z in helix_2_updated_coordinates.tolist():
                                                                 /len(helix_1_best_fit_coordinates),z_sum/len(
                                                                                                                                                                                                                                                                                                                      len(helix_2_updated_coordinates),z_sum/len(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 of each helix (Angstroms) \n')
z = + uns^z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      closest-contacts between helices
                                                                                                                                                                                                                                                   =+ mns-z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        to match with
                                                                                                   helix_1_best_fit_coordinates)]
                                                                                                                                                                                                                                                 x_sum += x; y_sum += y;
                                                                                                                                                                                                                                                                                                                                                       helix_2_updated_coordinates)]
x_sum += x; y_sum += y;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           . Using this to generate closest
                                                                                                                                                                        y_sum=0; z_sum=0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          every 25th frame basis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         outfile = open(output_file,'w')
                                                                                                                                          #repeat for helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 between CA particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  helix crossing
                                                                                                                                                                            x_sum=0;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     generalized
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  print
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      print
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         frame for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               on an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  output_file):
     867
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             875
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              879
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          881
                                                                                                                                                 869
                                                                                                                                                                                                                                                                                             873
                                                                                                                                                                                                                      871
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      877
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        404
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Ö

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any
                            FGFR3
                                                                                                                                                                                                                 CA and resid 24:46 )" #select second GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            second
                                                                                                                                                                                                                                                                                                                                                                                                          current_helix_1_coordinates = current_helix_1_CA_selection.coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                             current_helix_2_CA_selection.coordinates
                                                                                                                                                             and resid 1:23 )" #select first GpA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                closest contact for each of the residues from the first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 contact distances for
                                                                              34:66 )" #select second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    quickly
                            first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            the
                                                                                                                                                                                                                                                                                                                                                                               helix_2_CA_selection) #select CA particles in second helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            of
                                                                                                                                                                                                                                                                                                                         helix_1_CA_selection) #select CA particles in first helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  current_helix_1_coordinates, current_helix_2_coordinates)
                            #select
                                                                                                                                                                                                                                                                                                current_helix_1_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                    current_helix_2_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #now use the built-in MDAnalysis C-implemented function to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            generate a list of all (CA-CA) distance between helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         any element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             the result from a numpy array to a python list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       contact_distances = MDAnalysis.distances.distance_array(
                                                                                                                                                                                                                                                                                                                                                                                                                                    () #get a numpy array with helix 1 CA coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         () #get a numpy array with helix 2 CA coordinates
                           1:33 )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   contact_distances.tolist()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #the resulting list is a nested list of the n
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           first selection with
                                                                                                                                                                                                                                                                      in universe_object.trajectory[::skip_frames]:
                            resid
                                                                                resid
                           and
                                                                                and
                                                                                                                                                               CA
                           CA
                                                                                CA
                                                                                                                                                                 name
                                                                                                                                                                                                                 " ( name
                             name
                                                                                   name
                                                                                                                                                                                                                                                                                                                                                                                                                                                             current_helix_2_coordinates =
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=
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=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           given residue on the
                                                                                                                                                                                                                     II
                                                                                                                                                                 II
                               П
                                                                                    II
                                                                                                                                                               helix_1_CA_selection
                                                                                                                                                                                                                  helix_2_CA_selection
                            helix_1_CA_selection
                                                                                helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       contact_distances =
system_name == 'FGFR3':
                                                                                                                                    system_name == 'GpA':
                                                                                                          FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #so, find the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           selection:
                                                          monomer
                                                                                                                                                                                                                                                  monomer
                                                                                                                                                                                             monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #convert
                                                                                                                                                                                                                                                                          ဌ
                                                                                                                                     elif
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                                   883
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                                                                                                                                                                                                                                                                                                                                                                                  405^{\frac{7}{52}}
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every
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          if line.split()[0] == '#format:': continue #skip the first line which
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 angle (degrees) || column 2:
                                                                                                                                                                                                                                    list_of_frame_angle_pairs.append([frame_number,helix_crossing_angle])
                                                                                                                                    frame
                                                                                                                                                                                                                                                                                                   #so now we have a nested list of [ [frame #, helix crossing angle], [...],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       only runs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     stuff which starts at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  closest contact distance],
                                                                                                                                   25th
                                                                                                                                        every
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               list_of_frame_closest_contact_pairs.append([frame_number,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       distance and
                                                                                                                                                                                                                                                                                                                                                                                                                                        closest_contacts_file = open('closest_contacts_full.out','r')
                                                                                                                                    #because
                                                                                                                                                                                                                                                                                                                                                                                                         #Open the closest contacts file (has data for every frame):
                                                                                                                                                                                                   helix_crossing_angle = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   outfile = open('distance_vs_crossing_angle.out','w')
                                                                                                                               frame_number = (int(line.split()[0])) * 25
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #the 'limiting' information is the crossing angle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       number that depends on interhelix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                outfile.write('#format: column 1: helix crossing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             closest_contact = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  closest CA-CA interhelical contact (A) \setminus n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              is a comment that starts with this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #so now we have a nested list of [ [frame #,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           frame_number = int(line.split()[0])
                              = open('done.xvg','r')
#Open the helix crossing file for reading
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           list_of_frame_closest_contact_pairs =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             for line in closest_contacts_file:
                                                                                                 for line in helix_crossing_file:
                                                                  list_of_frame_angle_pairs =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 closest_contact])
                                                                                                                                                                                                                                                                      helix_crossing_file.close()
                                  helix_crossing_file
                                                                                                                                                                      parsed
                                                                                                                                                                                                                                                                                                                                      [...], etc. ]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       strict frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                [...], etc.]
      913
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             \overset{5}{407}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                935
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columns. The input file in each subfolder is named 'frame_correlated_position
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      dealt with by the parent
                                                                                                                                                                                                      closest_contact_frame_number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       rmsd-fixed frame of helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        theta and frame # in data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      \#the first line of this file reads: \#Format: helix 2 COM x position (A)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         cartesian_file: #this
                                                                                                                                                                                                                                                                                                                                                                                                                                          x and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      format in a file for each dimer simulation directory. '''
                                                                                                                                    for closest_contact_frame_number, closest_contact_distance
 the 'searcher':
                                                                                                                                                                                                                                    outfile.write(str(helix_crossing_angle)
                                                                                                                                                                                                                                                                                                                                                                                                                                          contain
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helix 2 COM y position (A) || frame # (starts at 1 for MDA)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        script, so just have to parse the data, do the work, and
                                                                                                                                                                                                                                                                    closest_contact_distance) + ' \setminus n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #open the file that contains relevant helix 2 position data
                                                                                                                                                                                                                                                                                                     print helix_crossing_frame_number,
                                                                                                                                                                                                                                                                                                                                                                                                                                       that
                                                                                                                                                                                                                                                                                                                                       closest_contact_frame_number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     .out' and the overhead with changing directories is
                                                                   helix_crossing_angle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              end
                                                                                                                                                                                                       II
II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           with open('frame_correlated_position.out','r') as
                                                                                                                                                                                                                                                                                                                                                                                                                                   directory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix 2 in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     convert to a gnuplot-ready file with polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            at the
     ន្ត
                                                                                                                                                                                                    if helix_crossing_frame_number
                                                                                                                                                                    list_of_frame_closest_contact_pairs
 list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           you won't need an explicit file.close()
helix crossing
                                                                                                                                                                                                                                                                                                                                                                                                                                     ''Scrape through the files in the data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            cartesian_coordinate_frame_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            cartesian_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        coordinates for the position of
                                                                   for helix_crossing_frame_number,
                                                                                                   list_of_frame_angle_pairs:
   the
                                                                                                                                                                                                                                                                                                                                                                                                     cartesian_to_polar_theta():
      so use
 frame,
                                                                                                                                                                                                                                                                                                                                                                                                            943
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cartesian_coordinate_frame_list.append([x_cartesian,y_cartesian
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             polar_file.write(str(element[0]) + ' ' + str(element[1]) + '\n
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              polar_file.write('#Format: column 1: frame # || column 2: polar theta
                                                                                                                                                                                                                                                                                                                                                                                                                                      #convert to a nested list that replaces x and y rectangular coordintaes with
                                           line which starts with the string '#Format:' as the first
if line.split()[0] == '#Format:' : continue #skip the first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    print element[0], element[1] #just so I can see it running
                                                                                                                                                                                                                                                                                                                                                          a nested list with format: [ [x_cartesian, y_cartesian, frame]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for helix 2 in rmsd-fixed frame of helix 1 (radians) \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          open('polar_interface_theta.out','w') as polar_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #write the polar data to a gnuplot-ready output file:
                                                                                                                   x_cartesian = float(line.split()[0])
                                                                                                                                                           y_cartesian = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             the corresponding polar coordinate angle theta:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           for x,y,frame in cartesian_coordinate_frame_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       polar_list.append([frame,polar_theta])
                                                                                                                                                                                               frame = int(line.split()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 polar_theta = math.atan2(y,x)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for element in polar_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       correlate_helixcrossing_polar_theta():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           the terminal
                                                                                                                                                                                                                                                                            frame])
                                                                                                                                                                                                                                                                                                                                                                                                [...], [...]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     polar_list = []
                                                                                                                                                                                                                                                                                                                                                              #so, now we
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               \frac{5}{409}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           971
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parse the
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take the helix crossing data (every 25th frame) in the
                                   the
                                                                                                                                                                                                                                                                                                                                                                                          every 25th
                                                                                                     match by frame, and output helix crossing angle and the corresponding polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_cross_list.append([frame_number, helix_crossing_angle])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        line
                                                                                                                                         certesians) to a new gnuplot-ready file.
                                                                   frame_correlated_position_noskip.out' files, determine which data points
                                   every frame in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         a hash on the first line of the frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #helix_cross_list is now a nested list: [ [frame_number, crossing angle],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        first
                                                                                                                                                                           Directory switching overhead is dealt with in parent script so just
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 position_file
                                                                                                                                                                                                                                                                                                                                                                                     frame_number = (int(line.split()[0])) * 25 #because
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  polar_theta = math.atan2(y_coordinate,x_coordinate)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      continue #skip the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    theta_list.append([frame_number, polar_theta])
                                                                                                                                                                                                                                                                                                                                                                                                                                                          helix_crossing_angle = float(line.split()[1])
                                   data calculated for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               when parsing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 with open('frame_correlated_position_noskip.out','r') as
                                                                                                                                                                                                           files and write the output file in this function.'''
                                                                                                                                                                                                                                                                                                                                                                                                                          frame parsed by helix crossing script
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        the file which contains a comment
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            y_coordinate = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          x_coordinate = float(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            frame_number = int(line.split()[2])
                                                                                                                                                                                                                                                                                                                   with open('done.xvg','r') as helix_crossing_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if line.split()[0][0] == '#' :
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             adjust for that
                                                                                                                                                                                                                                                                                                                                                        for line in helix_crossing_file:
                                   'done.xvg' files and the polar theta
                                                                                                                                                                                                                                                 data in a list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #there is a comment that starts with
                                                                                                                                       theta value (calculated from the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for line in position_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             correlated position file so
'''This function should
                                                                                                                                                                                                                                             #put the helix crossing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  [...], [...], etc. ]
                                                                                                                                                                                                                                                                                    helix_cross_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           theta_list =
        973
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         676
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   410^{\frac{7}{8}}
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intended
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                (Angstroms) || closest contact by residue helix 2 (Angstroms)\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               p, I
                                                                                                                                                                                                                                                                                                                                                                              (degrees)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  with open('FGFR3_heterodimer_replicate_7_frame_3271.out','w') as outfile:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        modification to a previous function that deals with finding the single
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Very minor
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              are
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               simulations that
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   are
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   closest_approach_representative(folder_name, universe_object, skip_frames
                                                                                                                                                                                                                                                    matched_data_list.append([helix_frame_number,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    + ' ' + str(element[1])
 [frame_number, polar theta],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             to compile a list of CA-CA contact distances for. These frames
                                                                                                                                                       in helix_cross_list:
                                                                                                                                                                                                                                                                                                                                                                          outfile.write('#format: frame # || helix crossing angle
                                                                                                                                                                                                                                                                                                                                          ,'w') as outfile:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          candidates for the starting points of atomistic simulations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     to represent different GpA or FGFR3 helix dimer interfaces.
                                                                                                                                                                                                                                                                                     helix_crossing_angle, polar_theta])
                                                                                                                                                                                                                      if helix_frame_number == polar_frame_number:
                                                                                                                                                                                        for polar_frame_number, polar_theta in theta_list:
                                                                                              data
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      element[2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ''I have a set of pdb structures (frames) from a few
                                                                                            frame-matched
                                                                                                                                                                                                                                                                                                                                             open('theta_helix_crossing_correlate.out',
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     print element[0], element[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   outfile.write(str(element[0])
                                                                                                                                                   for helix_frame_number, helix_crossing_angle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  str(element[2]) + ' n')
                                                                                                                                                                                                                                                                                                                                                                                                                                          matched_data_list:
list: [
                                                                                                                                                                                                                                                                                                               #make an output file and write the data
                                                                                              out
                                                                                         pick
                                                                                                                                                                                                                                                                                                                                                                                                         polar theta (radians) \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   distance.""
                                                                                              and
 a nested
                                                                                           #iterate through the lists
                                                                                                                                                                                                                                                                                                                                                                                                                                          for element in
                                                                                                                         matched_data_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      closest approach
 #theta_list is now
                                  [...], etc.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 system_name):
                                                                                                                                                                                                                                                                                                                                                                                                                                     411
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1011
         993
                                                                                                    995
                                                                                                                                                                                                                                                                                                                                                                                     1003
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1007
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     1009
                                                                                                                                                                997
                                                                                                                                                                                                                            666
                                                                                                                                                                                                                                                                                                                         1001
```

```
contact_distances_helix_1 = MDAnalysis.distances.distance_array
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             array with helix 1 CA coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  array with helix 2 CA coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_2_CA_selection) #select CA particles in second helix
                                                                                                                                the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  (current_helix_1_coordinates, current_helix_2_coordinates)
                                                                                                                                                                                                                                                                                                                                                                   in universe_object.trajectory[3270:3271:skip_frames]: #adjust
                                                                                                                                                                                                                                                             24:46 )" #select
                                                                                                                                                                                                                                                                                                                                                                                           for the specific frame/pdb file (MDA index is -1 relative to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_1_CA_selection) #select CA particles in first helix
                          #select
                                                                                                                                                                                                           and resid 1:23 )" #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                 current_helix_1_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         current_helix_2_CA_selection.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   current_helix_2_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     current_helix_1_CA_selection.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            function to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    a list of all (CA-CA) distance between
                                                                                                                                 generate
                                                                                                                                                                                                                                                                                                               native_adjustment = 72 #this number is used to generate
                                                                                                                                                                                                                                                                                                                                           native primary sequence numbering in the output
                                                                            34:66
                                                                                                                                                          the output
                         1:33
                                                                                                                                to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #now use the built-in MDAnalysis C-implemented
                                                                                                                              is used
                         resid
                                                                                                                                                                                                                                                               and resid
                                                                           resid
                                                                                                                                                          in
                         and
                                                                           and
                                                                                                                                #this number
                                                                                                                                                           sequence numbering
                                                                                                                                                                                                                                                               " ( name CA
                                                                                                                                                                                                        = "( name CA
                          CA
                                                                            CA
                           name
                                                                             name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               coordinates() #get a numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                coordinates() #get a numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       current_helix_1_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         current_helix_2_coordinates
                         _
=
                                                                                                                              = 366
                                                                                                                                                                                                                                                                 II
                                                                            helix_2_CA_selection =
                             II
                                                                                                       second FGFR3 monomer
                                                  first FGFR3 monomer
                                                                                                                                                                                                                                                                                        second GpA monomer
                       helix_1_CA_selection
                                                                                                                                                                                                           helix_1_CA_selection
                                                                                                                                                                                                                                                            helix_2_CA_selection
                                                                                                                                                                                                                                       first GpA monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     quickly generate
                                                                                                                                                                                 system_name == 'GpA':
'FGFR3'
                                                                                                                                 native_adjustment
                                                                                                                                                           native primary
   II
II
 system_name
                                                                                                                                                                                                                                                                                                                                                                                                                        frame)
                                                                                                                                                                                                                                                                                                                                                                      for ts
                                 1013
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```

```
'''Take the contents of the 'closest_contacts.out' and 'absolute_relative_Z.out
                                                                                                                                                                                                                                                                                                                                                                                                                                         Z coordinates, into a single gnuplot-ready file.'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       absolute
                                                                                                                                                                                                                                                                                                                                                                                                           absolute difference between
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   frame_contact_list.append([frame, closest_contact])
    line
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             line
                                                                                                                                                                                                                          + str(
if line.split()[0][0] == '#' : continue #skip
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          : continue #skip
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  output_file.write('#format: frame # || closest contact (A)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    with open('correlate_Z_closest_approach.out','w') as output_file:
                                                                                                                                                                                                                     output_file.write(str(frame_number) + '
                                                                                            helix_1_Z_coord = float(line.split()[1])
                                                                                                                          = float(line.split()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     closest_contact = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 difference in helical geo center Z coordinate (A)\n'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               with open('closest_contacts.out','r') as contacts_file:
                                                                                                                                                     abs(helix_1_Z_coord
                                                                                                                                                                                                                                                                                  print frame_number, delta_Z_absolute
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 with open('absolute_relative_Z.out','r') as Z_file:
                                                           frame_number = line.split()[0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       if line.split()[0][0] == '#'
                                                                                                                                                                                                                                                    delta_Z_absolute) + ' \langle n' \rangle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      frame = int(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                            closest contacts, and
                                                                                                                                                                                     helix_2_Z_coord)
                                                                                                                                                        delta_Z_absolute =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              for line in contacts_file:
                                    it is a comment
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             it is a comment
                                                                                                                          helix_2_Z_coord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           frame_contact_list = []
                                                                                                                                                                                                                                                                                                                                              absolute_delta_Z_and_closest_approach():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              for line in
                                                                                                                                                                                                                                                                                                                                                                                                        ' files and put frame #,
                                                                                                                                                                                                                                                                                                                                                                                                                                            geometric center
                                                                                                                                                                                                                                                                                                                                                                                                                                         helical
                                                                                                                                                                                                                                                                                                                                                 def
                                                                                                                                                                                                                                                                                                                                                     1053
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```

```
''This function should read in the 'relative_Z.out' file and split it into two
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    closest-contact distance less than 6
                                                                                                                                                                                                                                                                                                                                                                                                                                  new files, one pre- and the other post- dimerization, based on the frame at
     line if
                                                                                                                                             frame_and_contact_list,Z_value in zip(frame_contact_list,Z_list):
                                                                                                                                                                           output_file.write(str(frame_and_contact_list[0]) + ' ' + str(
    frame_and_contact_list[1]) + ' ' + str(Z_value) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              the
                                                                                                                                                                                                                                                  print frame_and_contact_list[0], frame_and_contact_list[1],
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  , folder_name,'; dimer start frame #: ', dimer_start_frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #determine the point at which the dimer forms (defined as closest-contact
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    correct
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            dimer_start_frame = int(line.split()[0]) #assign
     : continue #skip
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if line.split()[0][0] == '#' : continue #skip line if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              dimer start frame when the distance is
                                                                     absolute_delta_Z = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               with open('closest_contacts.out','r') as contacts_file:
                                                                                                           Z_list.append(absolute_delta_Z)
if line.split()[0][0] == '#'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Angstroms in the file 'closest_contacts.out').'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       float(line.split()[1]) < 6.0:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    which dimerization starts (defined as a
                                         it is a comment
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for line in contacts_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          a progress check
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          distance under 6 Angstroms)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  break
                                                                                                                                                                                                                                                                                    Z_value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            comment
                                                                                                                                                                                                                                                                                                                                                            split_Z_file(folder_name):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  print 'folder
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #print
                                                                                                                                                                                                                                                                                                                                                               def
                                                                                                                                                                                                                                                                                                                                                                     1075
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```
#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         the helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     lists for
#make some lists for storing the helix 1 and helix 2 values for pre- and post-
                                                                                                                                                                                                                                                                                                                                                                                                     relative to when
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        if int(line.split()[0]) >= dimer_start_frame:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           post_dimer_helix_1_Z_list.append(line
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      : continue #skip
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      if the system has reached or exceeded the
                                                                                                                                                                                                                                                       with open('post_dimer_Z_correlation.out','w') as post_dimer_Z_file:
                           simulation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #also split the line and dump
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  1 and helix 2 Z values to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           post_dimer_Z_file.write(line)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             usage later in correlation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 frame where dimerization occurs
                           can get a correlation coefficient for each
                                                                                                                                                                                                                                                                                  with open('pre_dimer_Z_correlation.out','w') as
                                                                                                                                                                                                                                                                                                                                                                                                    #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   if line.split()[0][0] == '#'
                                                                                                                                                                                                                                                                                                                                                                                                    frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 line if it is a comment
                                                                                                                                                                                                                                                                                                                                                                                                    #write the new files based on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                 dimerization starts:
                                                                                                                                                                                                                                                                                                                                                                                                                                                           for line in Z_file:
                                                                                                                                                                                                                             with open('relative_Z.out','r') as Z_file:
                                                                                                                                                                                                                                                                                                            pre_dimer_Z_file:
                                                      before and after dimerization:
                                                                                   pre_dimer_helix_1_Z_list = []
                                                                                                                                                                      post_dimer_helix_2_Z_list
                                                                                                                                          post_dimer_helix_1_Z_list
                                                                                                              pre_dimer_helix_2_Z_list
                           so I
                            dimerization
     1087
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     1101
                                                                                                                                                                                                                                                                                                                                                                                                     416
```

```
print 'Post-dimer R:',post_dimer_R_value.tolist()[1][0]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #write the values to the end of the appropriate files:
post_dimer_helix_2_Z_list.append(line.
                                                                                                                                                                                                                     pre_dimer_helix_2_Z_list.append(line.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   print 'Pre-dimer R:', pre_dimer_R_value.tolist()[1][0]
                                                                                                                                                        pre_dimer_helix_1_Z_list.append(line
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          of simulation box size as the simulation proceeds. At
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        and print out unit cell
                                                              yet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        grab the
                                                                                                                                                                                                                                                                                                                                                                                                              post\_dimer\_R\_value = numpy.corrcoef(numpy.array(
                                                                                                                                                                                                                                                                                 correlation coefficients using numpy
                                                                                                                                                                                                                                                                                                                   pre_dimer_R_value = numpy.corrcoef(numpy.array(
                                                              dimerized
                                                                                                                       pre_dimer_Z_file.write(line)
                                                                                                                                                                                                                                                                                                                                                                                                                                         post_dimer_helix_1_Z_list),numpy.array(
post_dimer_helix_2_Z_list))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                post_dimer_Z_file.write('R_value ' + str(
                                                                                                                                                                                                                                                                                                                                              pre_dimer_helix_1_Z_list), numpy.array(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           pre_dimer_Z_file.write('R_value ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              {\tt post\_dimer\_R\_value.tolist()[1][0]))}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            pre\_dimer\_R\_value.tolist()[1][0])
                                                            else: #if the system hasn't
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #convert numpy arrays back to lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        appropriate R values by indexing:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            def box_size_assessment(folder_name, universe_object, skip_frames):
                                                                                       farther than 6 A apart)
                                                                                                                                                                                   split()[1])
                                                                                                                                                                                                                                                  split()[2])
                            split()[2])
                                                                                                                                                                                                                                                                                                                                                                              pre_dimer_helix_2_Z_list))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          moment, simply iterate through the trajectory
                                                                                                                                                                                                                                                                                   #calculate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          an idea
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             getting
                                                                                                                                                                                                                                                                                                                                                                                                                      1113
                                                                      1107
                                                                                                                                                                                                                                                                                           1111
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                                                                                                                                                                                                                                                                                                                                                                                                                                              417
```

```
data folders are
                                                                                                                                                                                                                                                                                                                                                                                                                              sidekick_dimer_batch_analysis/closest_contact_results/' + folder_name.replace
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            FGFR3
                                                                                                                                                                                                                                                        Basically
                                                                                                                                                                                                                                                                                         write the
                                                                                                                                                                                                                                                                                                                 can't
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      precedes the
                                                                                                                                                                                                                                                                                                                                                                                                                                                         ('/','') + output_file #this should make the file names unique because the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            approach
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       second
                                                                                                                                                                                                                                                                                                              data in the separate local folder for FGFR3 SIDEKICK results because I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    and resid 1:23 )" #select first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              first
                                                                                                                                                                                                 def closest_contacts_efficient_SIDEKICK(folder_name, universe_object, skip_frames
                                                                                    str(universe_object
                                                                                                                                                                                                                                                        'August 29/ 2010: Modified version of the similarly named function.
                                                                                                                                                                                                                                                                                         t o
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   34:66 )" #select
                                                                                                                                                                                                                                                                                                                                                                       stored (at least not remotely when executing this from home).'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #select
                                                                                                                                                                                                                                                                                   the only difference here is that I have designed this function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      (folder name)
                                                                                                                                                                                                                                                                                                                                           write files to the mount where the large number of replicate
                                                                                                                                                                                                                                                                                                                                                                                                   output_file_path = '/sansom/sc2/bioc1009/Documents/FGFR3_work/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         2:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            1:33 )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        outfile.write('#format: column 1: frame number || column
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   between CA particles of each helix (Angstroms)\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      the full path on the mount
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   and resid
                                                                                   +
-
-
+
                                                       in universe_object.trajectory[::25]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              and
                                                                                    outfile.write(str(ts.frame)
                                                                                                                                            print folder_name, ts.frame
                            outfile:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      " ( name CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    CA
                                                                                                              trajectory.ts) + ' \setminus n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           "( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   "( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            outfile = open(output_file_path,'w')
                            with open('box_sizes.out','w') as
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               II
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_1_CA_selection
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 generic output_file name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               system_name == 'FGFR3':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           system_name == 'GpA':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      '/'-stripped string of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               FGFR3 monomer
                                                                                                                                                                                                                              system_name, output_file)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            monomer
dimensions.''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            elif
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```
contact distances for any
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      second
                                                                                                                                                                                                          current_helix_1_CA_selection.coordinates
                                                                                                                                                                                                                                                                 current_helix_2_CA_selection.coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       frame
and resid 24:46 )" #select second
                                                                                                                                                                                                                                                                                                                                quickly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    given
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   outfile.write(str(ts.frame) + ' ' + str(closest_contact_overall)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 the
                                                                                                                                                                           helix_2_CA_selection) #select CA particles in second helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        of
                                                                                                                    helix_1_CA_selection) #select CA particles in first helix
                                                                                                                                                                                                                                                                                                                                                                                                                    current_helix_1_coordinates, current_helix_2_coordinates)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               from
                                                                                       current_helix_1_CA_selection = universe_object.selectAtoms(
                                                                                                                                                current_helix_2_CA_selection = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                             #now use the built-in MDAnalysis C-implemented function to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            ಡ
                                                                                                                                                                                                                                                                                                                                                        generate a list of all (CA-CA) distance between helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                min(closest_contacts_by_residue)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     first selection with any element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       between helices in
                                                                                                                                                                                                                                                                                                                                                                                                                                                 a numpy array to a python list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         map(min, contact_distances)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                      contact_distances = MDAnalysis.distances.distance_array(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 the residues
                                                                                                                                                                                                                                       () #get a numpy array with helix 1 CA coordinates
                                                                                                                                                                                                                                                                                              #get a numpy array with helix 2 CA coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           contact_distances.tolist()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #the resulting list is a nested list of the n
                                                        in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                by finding the minimum of the above list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               οĘ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       contact
 CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 contact for
     name
                                                                                                                                                                                                          current_helix_1_coordinates =
                                                                                                                                                                                                                                                                current_helix_2_coordinates =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         closest_contacts_by_residue =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             closest_contact_overall =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                 #convert the result from
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      given residue on the
     II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #now find the overall
 helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            contact_distances =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               #so, find the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             selection:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        print
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```

#So, we now have the reference structure with center of geare are ready to start looping through frames in the traje	
))	
<pre>for ts in universe_object.trajectory[::skip_frames]:</pre>	
#Because helix 1 may move a bit we need to select	select it again in each
frame. We will also want to select helix 2 beca	2 because we are going to
track its relative position:	
<pre>current_helix_1_CA_selection = universe_object.selectAtoms</pre>	ect.selectAtoms(
helix_1_CA_selection)	
current_helix_2_CA_selection = universe_object.selectAtoms	$\mathtt{ect.selectAtoms}$ (
helix_2_CA_selection)	
#Find the (center of geometry zero'd) coordinates	inates for helix 1 in each
d	relative terms, perform the same
operation on helix 2 (i.e., translate it by the same	by the same amount):
current_helix_1_coordinates = current_helix_1_CA_s	current_helix_1_CA_selection.coordinates
() - current_helix_1_CA_selection.centerOfGeometry()	<pre>JfGeometry()</pre>
current_helix_2_coordinates = current_helix_2_CA_s	current_helix_2_CA_selection.coordinates
() - $current_helix_1_CA_selection.centerOfGeometry()$	<pre>JfGeometry()</pre>
ilgure out what kind of	ııs needed
d) fit of cu	erence st
This is based on the MDAnalysis/examples/rmsfit.py	/rmsfit.py example code
distributed with the most recent release of MDA	of MDAnalysis:
transformation = numpy.matrix(MDAnalysis.core.rms_fitting	re.rms_fitting.
rms_rotation_matrix(current_helix_1_coordinates,ref_coordinates	linates, ref_coordinates,
masses))	

```
center_of_mass_helix_2 = [x_sum/len(helix_2_updated_coordinates),y_sum/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     80
                                                                                                                                                                                                           all particles to represent the
#We don't actually need to apply the transformation to helix 1--just
                                                                                                                                                  Since the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               initialized in the head script
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #we want to extract the x and y coordinates for helix 2 such that they
                                                                                                                                                                                                                                                                                                                                                                                                                                                        code
                                                                                                                                                                                  ឧន
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  filtered by simulation type (i.e., folder name) and append them to
                              ٦.
S
                                                                                                                                                                                   mass
                                                                                                                                                                                                                                                                                                                                                                                                                                                     accessed by the
                                                                                                                                                  coordinates.
                                                          = current_helix_2_coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     exits
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           str(ts.frame)
                                                                                                                                                                              particles all have the same mass, we can treat the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             list_helix_2_centers.append(center_of_mass_helix_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                will bin the data after the frame-stepping loop
                               to 1 now
                                                                                                                                                                                                                                                                                                 for x, y, z in helix_2_updated_coordinates.tolist():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       in path_name:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                \mathtt{heterodimer\_helix\_2\_x\_coord\_list.append(x)}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              {\sf heterodimer\_helix\_2\_y\_coord\_list.append(y)}
                                                                                                                                                                                                                                                                                                                                                                                         len(helix_2_updated_coordinates),z_sum/len(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             can be appended as the function loops through)
                                                                                                                                                  of
                                                                                                                                                   matrix
                                                                                                                                                                                                                                                                                                                                                                                                                                                  a list so they can be
                              relative
                                                                                                                                                                                                                                                                                                                                 =+ wns-z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      frame: ' +
                                                                                                                                                                                                          average position of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           appropriately named list (these lists are
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  for (x,y,z) in list_helix_2_centers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR_______
                                                                                                                                                     a numpy
                              2 is
                                                                                                                                                                                                                                                                                                                                                                                                                  helix_2_updated_coordinates)]
                                                                                                                                                                                                                                                                                                                               x_sum += x; y_sum += y;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR_
                                                          helix_2_updated_coordinates
                            to know where helix
                                                                                                                                                represented as
                                                                                                                                                                                                                                                                      x_sum=0; y_sum=0; z_sum=0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        print str(path_name)
                                                                                                                                                                                                                                                                                                                                                                                                                                                   #put the centers in
                                                                                                                                                                                                        simply find the
                                                                                                                                                                                                                                         center of mass.
                                                                                            transformation
                                                                                                                                               #Helix 2 is
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1203
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```

1205	elif 'RRAGSVYAGILSYRVGFFLFILVVAAVTLCRLR
	rs:
1207	ant_helix_2_x_coord_list
1209	mutant_nellx_2_y_coora_list.appena(y) elif 'RRAGSVYAGILSYGVGFFLFILVVAAVTLCRLR
	ILSYGVGFFLFILVV,
1211	wildtype_helix_2_x_coord_list.append(x)
	list
1213	
	#so, after going through all 286 FGFR3 SIDEKICK dimer simulations, each of the
	lists will contain the approrpiate set of coordinates from 10 simulations.
	For binning and probability calculation it will be worthwhile to generalize
42	of the code that deals with that in a function and th
3	after this function from the head script
1215	
	def thermal_bins_SIDEKICK(helix_2_x_coord_list, helix_2_y_coord_list, outfile_path):
1217	'''Parses the FGFR3 helix 2 coordinate list data from the
	fixed_helix_thermal_merged() function and writes the binned probability data
	k with the *overall* FG
	position data, so the lists contain coordinates from all replicates in each
	of the three simulation conditions. Call this function after
	fixed_helix_thermal_merged() (and outside of main) in the head script. Note
	that the non-merged function is different in that it contains its own binning
	routines so you don't have to call this.'''
1219	outfile = open(outfile_path, 'w')
	#set a bin separation and build bins around min and max values
1221	bin_separation = 0.4

```
appropriate x-bin for the x coordinate and we need to iterate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if helix_2y > (y_bin - (bin_separation/2.0))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    and helix_2_y <= (y_bin + (bin_separation))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  if helix_2_x > (x_bin - (bin_separation/2.0)) and helix_2_x
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #if the above condition is satisfied then we have found the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for y_bin in y_bin_value_list: #the x,y coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     every x coordinate of helix 2 find a matching x-bin where it belongs:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for helix_2_x,helix_2_y in zip(helix_2_x_coord_list,helix_2_y_coord_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      through the y bins to see where the y coordinate fits:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         the x,y bin values in a tuple
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     coordinate falls in a 2D bin,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       +every time the helix 2 (x,y)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    should match because lists are ordered
                                   data
                                    οĮ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    (x_bin + (bin_separation/2.0)):
                                   based on the bounds
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    for x_bin in x_bin_value_list:
                                                                                                                                                                                                                                                                                                                                  while y <= max(helix_2_y_coord_list):</pre>
                                                                                                                                  while x <= max(helix_2_x_coord_list):</pre>
                                                                                                                                                                                                                                                                                                                                                                y_bin_value_list.append(y)
                                                                                                                                                                   x_bin_value_list.append(x)
                                   #populate the bin value lists
                                                               x = min(helix_2_x_coord_list)
                                                                                                                                                                                                                                                                   y = min(helix_2_y_coord_list)
                                                                                                                                                                                                     x += bin_separation
                                                                                                                                                                                                                                                                                                                                                                                                   y += bin_separation
list_of_bin_counts = []
                                                                                                x_bin_value_list = []
                                                                                                                                                                                                                                                                                                 y_bin_value_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1243
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```
to pick up new universe objects, so just feed them in to the optimize_cutoff
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #the head script that calls this function will automatically switch directories
                                              list_of_bin_counts.append((x_bin,y_bin)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  MDAnalysis tool for optimizing this cutoff for the selection of two leaflets
                                                                                                                                                                           sensible to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      gnuplot:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ''Oct. 7/ 2010: Adapting for use with FGFR3 simulations. A distance cutoff is
                                                                                                                                                                                                                                                                                                                                                                                                                                       outfile.write(str(x_bin) + ' ' + str(y_bin) + ' ' + str(float(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          leaflet selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            molecules belonging to a given leaflet. The purpose of this function is to
                                                                                                                                                                                                                    a gnuplot-ready probability for each 2d bin
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    call Mdanalysis.analysis.leaflet.optimize_cutoff(), which is a built-in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              rather than establishing extra networks or having cutoff problems when
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        list_of_bin_counts.count((x_bin,y_bin)))/float(len(
helix_2_x_coord_list))) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                an empty line between the blocks of (x-bin) data for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     used by MDanalysis.analysis.leaflet.LeafletFinder() to establish
                                                                                                                                                                             should be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             optimize_cutoff = MDAnalysis.analysis.leaflet.optimize_cutoff
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            function along with the necessary parameters for testing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               optimize_leaflet_selection_cutoff(folder_name, universe_object):
append to a list:
                                                                                                                                                                        sorted by default so it
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   straight to the LeafletFinder() routine.'''
                                                                                                                                                                                                                                                                                                                                                      for y_bin in y_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   import MDAnalysis.analysis.leaflet
                                                                                                                                                                           #the x and y bin lists should be
                                                                                                                                                                                                                       loop through each and produce
                                                                                                                                                                                                                                                                                                          for x_bin in x_bin_value_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                outfile.write('\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #we want
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       outfile.close()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    def
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       425
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        1259
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                                                                                                                                                                                                                                                                           1247
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$
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<pre>ystem_name == 'FGFR3': helix_1_CA_selection = " monomer helix_2_CA_selection = " FGFR3 monomer system_name == 'GpA': helix_1_CA_selection = " monomer helix_2_CA_selection = " monomer</pre>	the cutoff parameter is for the MDAnalysis network selection of leaflets and the optimal value varies for WT and hetero/mutant FGFR3 conditions	changes without worrying about which particles get selected as the distorts. Oct 5/2010: put in various print checks for testing; lo fixed the problem at check 3a and now commenting out the print che nition is the allowable distance (in Angstroms) between phosphate as and protein CA residues for inclusion in the 'local lipid shell parameter is for the MDAnalysis network selection of leaflets and limit value varies for WT and hetero/mutant FGFR3 conditions name == 'FGFR3': lix_1_CA_selection = "(name CA and resid 1:33)" #select first FG monomer lix_2_CA_selection = "(name CA and resid 34:66)" #select first Gp monomer lix_1_CA_selection = "(name CA and resid 24:46)" #select second monomer lix_2_CA_selection = "(name CA and resid 24:46)" #select second thickness near second monomer thickness near first monomer thickness near second monomer distal thickness (Angstroms)\n' thickness near second monomer distal thickness (Angstroms)\n' port MDAnalysis.analysis.leaflet se the same selection string used for optimizing the cutoff: osphate_selection = 'resname POP* and name P*' energale the leaflet selections from the first frame using the buill	- - - - - - - - - - - - - - - - - - -
the cutoff parameter is for the MDAnalysis network selection of leaflets the optimal value varies for WT and hetero/mutant FGFR3 conditions		the problem at check 3a and now commenting out the print is the allowable distance (in Angstroms) between phospha protein CA residues for inclusion in the 'local lipid sh	1277
local_definition is the allowable distance (in Angstroms) between phospha particles and protein CA residues for inclusion in the 'local lipid shother cutoff parameter is for the MDAnalysis network selection of leaflets the optimal value varies for WT and hetero/mutant FGFR3 conditions	local_definition is the allowable distance (in Angstroms) between phosp particles and protein CA residues for inclusion in the 'local lipid	changes without worrying about which particles get selected as distorts. Oct 5/2010: put in various print checks for testing; fixed the problem at check 3a and now commenting out the print	

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Or
                                                                                                                                                   the 'top
= MDAnalysis.analysis.leaflet.LeafletFinder(universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            (len(bottom_leaflet_phosphates
                                                                                                                                                                                                                                                                            and
                                                                         the 'bottom'
                                                                                                  to VMD shown
                                                                                                                                                                                                                                                                                                                                                                                                                            for phosphate_residue_number in bottom_leaflet_phosphates.resids()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               element
                                                                                                                                                                                                                          leaflets
                                                                                                                                                                                                                                                                           spaces
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                phosphate_residue_number in top_leaflet_phosphates.resids():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       bottom_phosphate_list.append(bottom_phosphate_string)
                                                                                                                                                                                                                                                    ρΛ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    resids())-1): #for all but the last resid element
                                                                                                                                                  this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              str(
                                                                                                                                                                                                                                                   parsed
                                                                                                                                                                                                                                                                           adding in
                                                                                                                                                                                                                          #Now I want python lists of phosphate residues for these
                                                                       bottom_leaflet_phosphates = leaflets.atoms(0) #clearly
                                                                                                                                                  #therefore,
                                                                                               system using the selection conversion
                                               out from the object:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          a right ')' after the last
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     bottom_phosphate_string = 'resid' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                     if bottom_leaflet_phosphates.resids().index(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     phosphate_residue_number) + ' or
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             bottom_phosphate_string = 'resid'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             phosphate_residue_number) + ' )'
                                                                                                                                                                                                                                                   ре
                                                                                                                                                                                                                                                  method) to be converted so they can
                                                                                                                                                                                                                                                                           Basically
                                                                                                                                                 top_leaflet_phosphates = leaflets.atoms(1)
                                                                                                                                                                        phosphates (top leaflet)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             v
                                                                                                                                                                                                                                                                             parser.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            phosphate_residue_number)
                                                 selections
                         phosphate_selection, cutoff)
                                                                                                                                                                                                                                                                           MDAnalysis u.selectAtoms()
                                                                                                                                                                                                                                                                                                                           #we'll want
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #repeat for top leaflet:
                                                                                                                                                                                                                                                                                                                               II
                                                 #pull the two leaflet
                                                                                                                                                                                                                                                                                                                           bottom_phosphate_list
                                                                                                                                                                                                                                                                                                                                                    top_phosphate_list =
                                                                                                                                                                                                                                                                                                                                                                                                    #for bottom leaflet:
                                                                                                    my
                                                                                                                                                                        (+Z) set of
                                                                                                  leaflet in
                                                                                                                                                                                                                                                resids()
                                                                                                                                                                                                                                                                                                     etc.:
 leaflets
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for
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s )" % (str(local_definition), helix_1_CA_selection) + ' and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   phosph_around_first_monomer_top_leaflet_string = "( around %s
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   leaflet within local_definition Angstroms of either protein
                                         phosphate_residue_number) < (len(top_leaflet_phosphates.
                                                                                                                                                                                                                        element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #I want to use MDAnalysis to parse for phosphates of each
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   bottom_phosphate_list) + ' and ( resname POP* and name P* )'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #Now that the 'absolute' leaflet selection strings are ready I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #selection strings for FGFR3 monomers for TOP leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        top_phosphate_list) + ' and ( resname POP* and name P* )'
                                                                                resids())-1): #for all but the last resid element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          bottom_leaflet_phosphate_selection_string = '(' + ''.join(
                                                                                                                                                                                                                 else: #we'll want a right ')' after the last resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             top_leaflet_phosphate_selection_string = '( ' + ''.join(
                                                                                                                                                                                                                                                                                                                                                top_phosphate_list.append(top_phosphate_string)
                                                                                                                                                                                                                                                    top_phosphate_string = 'resid ' + str(
   phosphate_residue_number) + ' )'
                                                                                                                            top_phosphate_string = 'resid' + str(
                                                                                                                                                                   phosphate_residue_number) + ' or '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          + top_leaflet_phosphate_selection_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for ts in universe_object.trajectory[::skip_frames]:
if top_leaflet_phosphates.resids().index(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         phosphates within local_definition:
                                                                                                                                                                                                                                                                                                                                                                                                                                     #assign the formatted selection strings:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       start the trajectory looping:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               molecule.
        1313
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selectAtoms(phosph_around_first_monomer_bottom_leaflet_string
s,
%
                                                                                                                                                                                                                                                                                                                                                                                 phosph_around_second_monomer_bottom_leaflet_string = "( around
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             selectAtoms(phosph_around_second_monomer_top_leaflet_string)
                                                                                                                                                                                                                                                          around
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        selectAtoms(phosph_around_first_monomer_top_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                                        %s %s )" % (str(local_definition), helix_2_CA_selection) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     phosph_around_first_monomer_bottom_leaflet = universe_object.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #will need separate selection strings for leaflet phosphates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 top_leaflet_phosphate_selection_string + ' and not ' + "(
phosph_around_second_monomer_top_leaflet_string = "( around
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   phosph_around_second_monomer_top_leaflet = universe_object.
                                                                                                                                                                                                                                                                                               s %s )" % (str(local_definition), helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                protein:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  = universe_object.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            bottom_leaflet_phosphate_selection_string + ' and not
                                          (str(local_definition), helix_2_CA_selection)
                                                                                                                                                                                                                                                        phosph_around_first_monomer_bottom_leaflet_string = "(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                and ' + bottom_leaflet_phosphate_selection_string
                                                                                                                                                                                                                                                                                                                                     and ' + bottom_leaflet_phosphate_selection_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     actual MDAnalysis AtomGroup selections
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     around %s ( name CA ) )" % str(local_definition)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                around %s ( name CA ) )" % str(local_definition)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              that are NOT within local_definition of either
                                                                                     top_leaflet_phosphate_selection_string
                                                                                                                                                                     within
                                                                                                                                                                       phosphates
                                                                                                                                                                                                              FGFR3 monomer 1 or
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                phosph_around_first_monomer_top_leaflet
                                                                                                                                                                     #same thing for bottom leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 bottom_distal_phosphate_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      top_distal_phosphate_string
                                                                                                                                                                                                                local_definition of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #produce the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                1343
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```
	exttt{phosph\_around\_first\_monomer\_top\_leaflet.centerOfGeometry()[2]}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              protein
universe_object.
                                                                                                                                                                                                                                                             measure
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  phosph_around_second_monomer_top_leaflet.centerOfGeometry()
                                                                                                                                                                                                                                                                                       of bilayer thickness) of the leaflet phosphate centers of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                either
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              str(ts.frame)
                                                                                                                                                                                                                                                         #Calculate the difference in the Z coordinate (i.e., a
                                                                                                                                                          = universe_object.selectAtoms(
                                                            phosph_around_second_monomer_bottom_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = abs(top_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               [2] - phosph_around_second_monomer_bottom_leaflet
                                                                                            top_distal_phosphate = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           bottom_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #everywhere else (not within local_definition of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   - phosph_around_first_monomer_bottom_leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 bilayer_Z_thickness_near_second_monomer = abs(
   II
                                                                                                                                                                                                                                                                                                                                                                                                                      abs (
phosph_around_second_monomer_bottom_leaflet
                                                                                                                                                                                                                                                                                                                          scenarios:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                +
                                                                                                                                                                                                                                                                                                                                                                                                                      bilayer_Z_thickness_near_first_monomer =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frame:
                                                                                                                                                                                           bottom_distal_phosphate_string)
                                                                                                                                                                                                                                                                                                                          a few different
                                                                                                                             top_distal_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #track progress on the prompt:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #near second FGFR3 monomer:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            bilayer_Z_thickness_distal
                                                                                                                                                                                                                                                                                                                                                                                      #near first FGFR3 monomer:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  centerOfGeometry()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               centerOfGeometry()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          centerOfGeometry()[2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             centerOfGeometry()[2]
                                                                                                                                                         bottom_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          print str(folder_name)
                                                                                                                                                                                                                                                                                                                      geometry for
                              selectAtoms
                                                                                                                                                                                                                                                                                                                                                             1349
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                                                                                                                                                                                                                                                                                                                                                                                                                                                       431
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lipids as the first 2-3 shells (~90 lipids) around rhomboid I want to see how
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      FGFR3
                                                                                                                                             outfile_name
                                                                                                                                                                                                                                                                                                                                                      above. Could be that my sample size is a bit small. Adjustments made on Oct
                                                                                                                                                                                                       '''Oct. 9/2010: Adapting this function for use with GpA and FGFR3 simulations
                                                                                                                                                                                                                                                                                           many lipids are being used to define 'local' based on the cutoff distance
                                                                                                                                                                                                                                Because White and co-workers (Structure 17, 395-405, 2009) define 'local'
                                                                                                                                                                                                                                                                                                                       parameter: local_definition) I've used in the analyze_leaflets() function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     CA residues for inclusion in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     "( name CA and resid 1:23 )" #select first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       local_definition is the allowable distance (in Angstroms) between
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 generation
                                                          str (
                             + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              34:66 )" #select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #select
                                                                                                                                                                                                                                                                                                                                                                                  5/ 2010 to match the repair to the phosphate selection issues in
                                                                                                                                             universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               the MDAnalysis network-leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   1:33 )"
                                                     bilayer_Z_thickness_near_second_monomer)
                          bilayer_Z_thickness_near_first_monomer)
+ str(
                                                                                   bilayer_Z_thickness_distal) + ' \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      and resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                resid
 +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                and
outfile.write(str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        and protein
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 name
                                                                                                                                                                                                                                                                                                                                                                                                               analyze_leaflets() function above.
                                                                                                                                             count_lipids_in_local_shell(folder_name,
                                                                                                                                                                           local_definition, cutoff, system_name):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    _
=
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 cutoff argument is for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       phosphate particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 local lipid shell'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_1_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        system_name == 'FGFR3':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       system_name == 'GpA':
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            FGFR3 monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           elif
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         if
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                                                                                                                                                                                                                                                                                                                                                                                                                   432
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```
phosphate_residue_number) < (len(bottom_leaflet_phosphates.
                                                                                                                                                                                                                                                                                              leaflets = MDAnalysis.analysis.leaflet.LeafletFinder(universe_object,
and resid 24:46 )" #select second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for phosphate_residue_number in bottom_leaflet_phosphates.resids():
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         phosphate_residue_number) < (len(top_leaflet_phosphates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for phosphate_residue_number in top_leaflet_phosphates.resids()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        bottom_phosphate_list.append(bottom_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  resids())-1): #for all but the last resid element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           else: #we'll want a right ')' after the last resid
                                                                                                                                                 helix1_bottom | helix2_top | helix2_bottom | distal_top
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  str(
                                                                                                              outfile.write('#format for lipid resid count: helix1_top
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      phosphate_residue_number) + ' or '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                bottom_phosphate_string = 'resid' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     bottom_phosphate_string = 'resid' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          if bottom_leaflet_phosphates.resids().index(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               phosphate_residue_number) + ' )'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    if top_leaflet_phosphates.resids().index(
                                                                                                                                                                                                                                                                                                                                                                      bottom_leaflet_phosphates = leaflets.atoms(0)
                                                                                                                                                                                                                                                                                                                                                                                                           top_leaflet_phosphates = leaflets.atoms(1)
                                                                                                                                                                                                                                                            selection = 'resname POP* and name P*'
                                                                                                                                                                                                                         import MDAnalysis.analysis.leaflet
   name CA
                                                                       open(outfile_name, 'w') as outfile:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #repeat for top leaflet:
         II
                                                                                                                                                                                                                                                                                                                                                                                                                                               bottom_phosphate_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 top_phosphate_list =
                                                                                                                                                                                                                                                                                                                                   selection, cutoff)
   helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #for bottom leaflet:
                                                                                                                                                                                 distal_bottom\n')
                                                                             with
           1371
                                                                                                                       1373
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1383
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```
#I want to find out how many lipids (although I'm parsing their
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               phosph_around_helix2_top_leaflet_string = "( around %s %s )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   phosph_around_helix1_top_leaflet_string = "( around %s %s )"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               (str(local_definition), helix_1_CA_selection) + ' and '
                                                                                                                                 element
                                                                                                                                                                                                                                                                                                                                                                                    #assign the formatted selection strings (Oct. 5/ 2010: adjusted to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    bottom_phosphate_list) + ' and ( resname POP* and name P* )'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #Now that the 'absolute' leaflet selection strings are ready I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      top_phosphate_list) + ' and ( resname POP* and name P* )'
resids())-1): #for all but the last resid element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        bottom_leaflet_phosphate_selection_string = '( ' + ''.join(
                                                                                                                             else: #we'll want a right ')' after the last resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           top_leaflet_phosphate_selection_string = '( ' + ''.join(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              (str(local_definition), helix_2_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 phosphates) are selected per analysis frame
                                                                                                                                                                phosphate_residue_number) + ' '' ' te_list_arran''.
                                                                                                                                                                                                                                                             top_phosphate_list.append(top_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               for ts in universe_object.trajectory[::skip_frames]:
                                                                                    phosphate_residue_number) + ' or
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          top_leaflet_phosphate_selection_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          top_leaflet_phosphate_selection_string
                                                                                                                                                                      top_phosphate_string = 'resid'
                                           top_phosphate_string = 'resid
                                                                                                                                                                                                                                                                                                                                                                                                                                    properly include phosphate specification):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        start the trajectory looping:
                                                                                                                                          1393
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     1399
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```
phosph_around_helix1_top_leaflet = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               phosph_around_helix2_top_leaflet = universe_object.selectAtoms(
                                                                                                                                              % (str(local_definition), helix_2_CA_selection) + ' and '
                                     + ' and '
phosph_around_helix1_bottom_leaflet_string = "( around %s %s
                                                                                                           " (around %s %s
                                                                                                                                                                                                                                                                                               selectAtoms(phosph_around_helix1_bottom_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 selectAtoms(phosph_around_helix2_bottom_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                            not
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #now I want a way to count the number of lipid 'residues'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         = universe_object.
                                                                                                                                                                                                                                                                                                top_leaflet_phosphate_selection_string + ' and not
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              phosph_around_helix2_bottom_leaflet = universe_object
                                                                                                                                                                                                                                                                                                                                                                                                          'and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      AtomGroup selections:
                                     (str(local_definition), helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                around %s ( name CA ) )" % str(local_definition)
                                                                                                                                                                                                                                                                                                                                                                                                                                          %s ( name CA ) )" % str(local_definition)
                                                                                                                                                                                                                                                                                                                                                                                                          bottom_leaflet_phosphate_selection_string +
                                                                                                             phosph_around_helix2_bottom_leaflet_string =
                                                                    bottom_leaflet_phosphate_selection_string
                                                                                                                                                                                     bottom_leaflet_phosphate_selection_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            phosph_around_helix1_top_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    phosph_around_helix2_top_leaflet_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     phosph_around_helix1_bottom_leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              bottom_distal_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      MDAnalysis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         top_distal_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                     bottom_distal_phosphate_string
                                                                                                                                                                                                                                                                 II
                                                                                                                                                                                                                                                          top_distal_phosphate_string
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              bottom_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      top_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      actual
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #produce the
                                                                                                                                                                                                                                                                                                                                                                                                                                               around
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       \frac{1}{4}35
           1409
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   1423
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1421
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in each case -- the .numberOfResidues() method should

selected

```
cutoff):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               an absolute definition of what
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          2010:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   To ensure that my bilayer thickness tracking algorithm is valid with this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          system I have to be able to show that there is no lipid flip-flop between
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       track the lipids from there.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               outfile.write(str(num_phosph_around_helix1_top_leaflet) +
                                                                                                                                                                                                                                          phosph_around_helix1_bottom_leaflet.numberOfResidues()
                                                                                                                                                                                                                                                                                                                       phosph_around_helix2_bottom_leaflet.numberOfResidues()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  outfile_name,
                                                                             phosph_around_helix1_top_leaflet.numberOfResidues()
                                                                                                                                                          phosph_around_helix2_top_leaflet.numberOfResidues()
                                                                                                                                                                                                                                                                                                                                                                                                                                             num_bottom_distal_phosphate = bottom_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  str (
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       str(num_phosph_around_helix1_bottom_leaflet) + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ''Oct. 11/ 2010: Adjusting for use with {\rm GpA/FGFR3} simulations; Oct.
                                                                                                                                                                                                                                                                                                                                                              num_top_distal_phosphate = top_distal_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     num_phosph_around_helix2_bottom_leaflet)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                num_phosph_around_helix2_top_leaflet) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    flip_flop_tracker(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                 num_phosph_around_helix1_bottom_leaflet
                                                                                                                                                                                                                                                                                num_phosph_around_helix2_bottom_leaflet
                                      num_phosph_around_helix1_top_leaflet
                                                                                                                  num_phosph_around_helix2_top_leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            num_top_distal_phosphate) + ' '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             frame because I use
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       lipid residue is in which leaflet and simply
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      num_bottom_distal_phosphate)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   #track progress on the prompt:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        print str(folder_name)
                                                                                                                                                                                                                                                                                                                                                                                                       numberOfResidues()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    numberOfResidues()
do the trick:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 leaflets after the first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      def
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              1435
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                                                                                                                              1425
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```
= MDAnalysis.analysis.leaflet.LeafletFinder(universe_object,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     phosphate_residue_number) < (len(bottom_leaflet_phosphates
                                                                                                                                                                                                                                                                                                                                                                                                                                                          select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           for phosphate_residue_number in bottom_leaflet_phosphates.resids()
   and
                                                                                                                                                                                                              select the leaflet
 selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  bottom_phosphate_list.append(bottom_phosphate_string)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   element
                                                                                                                                                                                                                                                                                                                                                                                                                                                         functions,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        str(
                                                                                                                                                   smallest_Z_top_phosphate | largest_Z_bottom_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           phosphate_residue_number) + ' or '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       else: #we'll want a right ')' after the last
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             bottom_phosphate_string = 'resid' +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 resids())-1): #for all but the last resid
leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      if bottom_leaflet_phosphates.resids().index(
                                                                                                                                                                              smallest_Z_bottom_phosphate |system_Z_center\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                         analysis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       bottom_phosphate_string = 'resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   -
+
                                                                                                                      outfile.write('#format: largest_Z_top_phosphate
                                                                                                                                                                                                           #use the built-in MDAnalysis leaflet module to
                                                                                                                                                                                                                                                                                                                                                                                            bottom_leaflet_phosphates = leaflets.atoms(0)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   phosphate_residue_number)
the networkx cutoff use by MDAnalysis for
                            WT, and FGFR3 hetero/mutant
                                                                                                                                                                                                                                             usual:
                                                                                                                                                                                                                                                                                                                                                                                                                          top_leaflet_phosphates = leaflets.atoms(1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                       #as I've done for the other two bilayer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    phosphate particles in each leaflet:
                                                                                                                                                                                                                                                                                                     -
*
스
                                                                                                                                                                                                                                          residues from the first frame as
                                                                                                                                                                                                                                                                                                       and name
                                                                                                                                                                                                                                                                       import MDAnalysis.analysis.leaflet
                                                                                      with open(outfile_name, 'w') as outfile:
                                                                                                                                                                                                                                                                                                     selection = 'resname POP*
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  bottom_phosphate_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  II
                                                                                                                                                                                                                                                                                                                                                                selection, cutoff)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              leaflet:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 top_phosphate_list
                            GpA, FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              bottom
                                                                                                                                                                                                                                                                                                                                    leaflets
                              for
٦.
S
                                  varies
cutoff
                                                                  1439
                                                                                                                                                                                                                                                                                                                                                                                                                            \overset{_{1}^{2}}{437}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          1457
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```
#produce a list of the Z coordinates for each phosphate in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #find the largest Z coordinate in the top leaflet phosphates:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #Now that the 'absolute' leaflet selection strings are ready I will
                                                                                                                              phosphate_residue_number) < (len(top_leaflet_phosphates.
                                                                                                                                                                                                                                                                                                    else: #we'll want a right ')' after the last resid element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       largest_Z_top_phosphate = top_phosphate_coordinates[0][2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           top_phosphate_coordinates = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    top_leaflet_phosphate_selection_string).coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #assign the formatted selection strings (Oct. 5/ 2010: adjusted
                                           for phosphate_residue_number in top_leaflet_phosphates.resids()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    bottom_phosphate_list) + ' and ( resname POP* and name P* )'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    top_phosphate_list) + ' and ( resname POP* and name P* )'
                                                                                                                                                                       resids())-1): #for all but the last resid element
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          bottom_leaflet_phosphate_selection_string = '( ' + ''.join(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           top_leaflet_phosphate_selection_string = '( ' + ''.join(
                                                                                                                                                                                                                                                                                                                                                                                                                                     top_phosphate_list.append(top_phosphate_string)
                                                                                                                                                                                                                                                                                                                                         phosphate_residue_number) + ')'
                                                                                                                                                                                                               top_phosphate_string = 'resid' + str(
                                                                                                                                                                                                                                                        phosphate_residue_number) + ' or '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               for ts in universe_object.trajectory[::skip_frames]:
                                                                                     if top_leaflet_phosphates.resids().index(
                                                                                                                                                                                                                                                                                                                                             top_phosphate_string = 'resid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   properly include phosphate specification):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     start the trajectory looping:
#repeat for top leaflet:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 top leaflet:
             1459
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    1477
                                                                                                                                                                                                                                                                                                                                                                                                                                             1465
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                                                                                               1461
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```
#find the largest Z coordinate in the bottom leaflet phosphates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   largest_Z_bottom_phosphate = bottom_phosphate_coordinates[0][2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   smallest_Z_bottom_phosphate = xyz_coordinate[2]
                                                                                                                                      #find the smallest Z coordinate in the top leaflet phosphates:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #produce a list of the Z coordinates for each phosphate in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #find the Z coordinate of the center of geometry of the entire
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     largest_Z_bottom_phosphate = xyz_coordinate[2]
                                                                                                                                                                                                                                                                                                                                smallest_Z_top_phosphate = xyz_coordinate[2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       bottom_phosphate_coordinates = universe_object.selectAtoms(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 if xyz_coordinate[2] < smallest_Z_bottom_phosphate:</pre>
                                                                                      = xyz_coordinate[2]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         bottom_leaflet_phosphate_selection_string).coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            if xyz_coordinate[2] > largest_Z_bottom_phosphate:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          smallest_Z_bottom_phosphate = bottom_phosphate_coordinates
                                                                                                                                                                                      smallest_Z_top_phosphate = top_phosphate_coordinates[0][2]
                                                                                                                                                                                                                                                                           xyz_coordinate[2] < smallest_Z_top_phosphate:
                                           if xyz_coordinate[2] > largest_Z_top_phosphate:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #find the smallest Z coordinate in the bottom leaflet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     for xyz_coordinate in bottom_phosphate_coordinates:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for xyz_coordinate in bottom_phosphate_coordinates:
for xyz_coordinate in top_phosphate_coordinates:
                                                                                                                                                                                                                                  for xyz_coordinate in top_phosphate_coordinates:
                                                                                                                                                                                                                                                                                                                                                                                                                              #repeat the process for the bottom leaflet:
                                                                                         largest_Z_top_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           bottom leaflet:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              phosphates:
                                                         1479
                                                                                                                                                                                                                                                                                                                                              1485
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1491
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439

1497

1499

1501

(phosphate) lipid system in this frame:

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FGFR3 hetereo, and FGFR3 mutant conditions. I would like to further subdivide
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           GpA_local_thickness_pre_dimer_list, GpA_local_thickness_post_dimer_list, folder_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     protein-local bilayer thickness to an average of the two helices before
                                                                                                                                                                                                                                                                                                         | + str(system_Z_center) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             -distal bilayer thickness results for easy comparison of GpA, FGFR3 WT
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 WT_FGFR3_local_thickness_pre_dimer_list, WT_FGFR3_local_thickness_post_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       '''Oct. 13/2010: Now I want averaged (with standard deviation) protein-local
P0P*
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             hetero_FGFR3_local_thickness_post_dimer_list, mutant_FGFR3_distal_thickness_list,
                                                                                                                    for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  hetero_FGFR3_distal_thickness_list, hetero_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     largest_Z_bottom_phosphate, smallest_Z_bottom_phosphate
                                                                                                                                                                                                                                                                                                                                                                                                                                                              #print largest_Z_top_phosphate, smallest_Z_top_phosphate,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     str(ts.frame)
                                                                                                                  check
                                                                                                                                                                                           str(
universe_object.selectAtoms('resname
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      mutant_FGFR3_local_thickness_post_dimer_list, GpA_distal_thickness_list,
                                                                                                                    can
                                                                                                                  Н
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             def bilayer_thickness_average_results(WT_FGFR3_distal_thickness_list,
                                                                                                                                                                                           +
                                                                                                                    80
                                                                                                                                                                                         outfile.write(str(largest_Z_top_phosphate)
                                                                                                                                                                                                                                                                     + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 + ' -- frame: ' +
                                                                                                                  file
                                                                                                                                                                                                                                                                                                                                                                                                                      #print out results in testing phase:
                                                                                                                  to
                                                                                                                                                                                                                                                                 largest_Z_bottom_phosphate) + '
                                    name P*').centerOfGeometry()[2]
                                                                                                                                                                                                                                                                                                             +
                                                                                                                  coordinates
                                                                                                                                                                                                                                                                                                       smallest_Z_bottom_phosphate)
                                                                                                                                                                                                                            smallest_Z_top_phosphate) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #track progress on the prompt:
                                                                                                                                                    lipid flip-flop activity:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  mutant_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             print str(folder_name)
                                                                                                                #print the various Z
system_Z_center =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            system_Z_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    dimerization_criterion):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            440^{\frac{6021}{1200}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                1513
                                                                                     1503
                                                                                                                                                                                                                                                                                                                                                                                                                                 1507
                                                                                                                                                                                                   1505
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```

and after dimerization.	Angstroms) for defining the frame as a 'dimer frame' where the trajectory is then split for the purposes of averaging pre- and post- dimer local bilayer thicknesses (around 6 Angstroms is probably reasonable)	I will use a separate function to calculate average and standard deviation of the global lists and print this to a file.		<pre>#to track progress: print folder_name, 'starting'</pre>	#check for condition based on folder name to decide which lists get appended t	if 'GpA' in folder_name:	local_pre_dimer_list = GpA_local_thickness_pre_dimer_list local_post_dimer_list = GpA_local_thickness_post_dimer_list	<pre>r' in folder_name: ist = hetero_FGFR3_distal_thickness</pre>	<pre>local_pre_dimer_list = hetero_FGFR3_local_thickness_pre_dimer_list local_post_dimer_list = hetero_FGFR3_local_thickness_post_dimer_list</pre>	<pre>elif 'wildtype' in folder_name: distal_list = WT_FGFR3_distal_thickness_list</pre>	<pre>local_pre_dimer_list = WT_FGFR3_local_thickness_pre_dimer_list local_post_dimer_list = WT_FGFR3_local_thickness_post_dimer_list</pre>	in folder_name:
1515		1517	1519	1521	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1525	1527	1529	1531	1533	1535	1537

```
protein dissociation events after dimerization so it'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           dimer frame: ',first_dimer_frame #tracking
                                                                                                                                                                                                                                                                                                                                                                                                                                                           has
                                                                                                                                                                     get access to the data that I need
                                                                 mutant_FGFR3_local_thickness_post_dimer_list
                                                                                                                                         simply need to
                                                                                                                                                                                                                                                                                                                if '#' in line: continue #ignore the line if it's a comment
                                                                                                                                                                                                                                                                                                                                                                                                                                                         dimer
                                 mutant_FGFR3_local_thickness_pre_dimer_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                my POPC simulations have
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                break #no need to continue parsing once the dimer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        s a clean split of the trajectory at this frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #now open the file that contains the protein-local and -distal bilayer
                                                                                                                                                                                                                                                                                                                                                                                                                                                              ಡ
                                                                                                                                                                                                                                             as closest_contact_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                         if closest_approach <= dimerization_criterion: #if</pre>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              with open('bilayer_thickness.out','r') as bilayer_thickness_file:
                                                                                                                                                                                                        assess the approximate frame at which dimerization occurs:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   if '#' in line: continue #ignore comment line(s)
                                                                                                                                                                                                                                                                                                                                               starting with a hash (i.e., the first line)
                                                                                                                                       directories so I
                                                                                                                                                                                                                                                                                                                                                                                                                  closest_approach = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            first_dimer_frame = frame_number
distal_list = mutant_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           number for averaging purposes
                                                                                                                                                                                                                                                                                                                                                                               frame_number = int(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   formed because none of
                                                                                                                                                                                                                                           with open('closest_contacts_full.out', 'r')
                                                                                                                                     care of changing
                                                                                                                                                                        open the closest approach output file to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for line in bilayer_thickness_file:
                                                                                                                                                                                                                                                                             for line in closest_contact_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print 'first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    progress
                                                                        II
                                      II
                                                                    local_post_dimer_list
                                 local_pre_dimer_list
                                                                                                                                       #the head script will take
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  thickness results:
                                           1539
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               1557
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                                                                                                              1541
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```

```
about
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  local_post_dimer_list.append(thickness_near_monomer_1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             local_post_dimer_list.append(thickness_near_monomer_2)
                                                                                                                                                                                                                                                                                                                                                             if frame_number < first_dimer_frame: #if dimerization has not
                                                                                                                                                                                                                                                                                                                                                                                                                                                local_pre_dimer_list.append(thickness_near_monomer_1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   WT_FGFR3_local_thickness_pre_dimer_list, WT_FGFR3_local_thickness_post_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             local_pre_dimer_list.append(thickness_near_monomer_2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             hetero_FGFR3_local_thickness_post_dimer_list, mutant_FGFR3_distal_thickness_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    bilayer_thickness_average_results() and generate stats, then output to a
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             the post-dimer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              hetero_FGFR3_distal_thickness_list, hetero_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              This is for tracking overall average protein-local and -distal bilayer
                                                                                                                                                                               I don't care
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            GpA_local_thickness_pre_dimer_list, GpA_local_thickness_post_dimer_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   mutant_FGFR3_local_thickness_post_dimer_list, GpA_distal_thickness_list,
                                                                                     = float(line.split()[2])
                                          = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        elif frame_number >= first_dimer_frame: #use
                                                                                                                                                                             #always append the distal thickness because
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     local list if dimerization has occurred
                                                                                                                                distal_thickness = float(line.split()[3])
                                                                                                                                                                                                                                                                     distal_list.append(distal_thickness)
frame_number = int(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           ''Take the data in the global lists populated by
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            thicknesses for GpA and FGFR3 conditions.'''
                                                                                                                                                                                                                            dimerization for that value:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           def bilayer_stats(WT_FGFR3_distal_thickness_list,
                                               thickness_near_monomer_1
                                                                                     thickness_near_monomer_2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        mutant_FGFR3_local_thickness_pre_dimer_list,
                                                                                                                                                                                                                                                                                                                                                                                                        yet occurred:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   print folder_name, 'done'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #to track progress:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     443
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      1575
                                                          1559
                                                                                                                                                                                                                                                                                                                                                                        1565
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         1567
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1573
                                                                                                                                                1561
                                                                                                                                                                                                                                                                                1563
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      1571
```

```
= numpy.std(mutant_FGFR3_local_thickness_post_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     _thickness_post_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  hetero\_local\_pre\_std = numpy.std(hetero\_FGFR3\_local\_thickness\_pre\_dimer\_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            mutant_local_pre_std = numpy.std(mutant_FGFR3_local_thickness_pre_dimer_list)
                                                                                                       numpy.average(WT_FGFR3_local_thickness_post_dimer_list)
                                                                      = numpy.average(WT_FGFR3_local_thickness_pre_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             GpA\_local\_post\_avg = numpy.average(GpA\_local\_thickness\_post\_dimer\_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            = numpy.std(WT_FGFR3_local_thickness_post_dimer_list)
                                                                                                                                                                                                                                                                                                                                = numpy.average(mutant_FGFR3_distal_thickness_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        GpA_local_pre_avg = numpy.average(GpA_local_thickness_pre_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     WT_local_pre_std = numpy.std(WT_FGFR3_local_thickness_pre_dimer_list)
                                                                                                                                            = numpy.average(hetero_FGFR3_distal_thickness_list)
lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          mutant_distal_std = numpy.std(mutant_FGFR3_distal_thickness_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         GpA_local_pre_std = numpy.std(GpA_local_thickness_pre_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               hetero_distal_std = numpy.std(hetero_FGFR3_distal_thickness_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #use numpy to calculate the corresponding standard deviations:
 thickness
                                 = numpy.average(WT_FGFR3_distal_thickness_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    GpA_distal_avg = numpy.average(GpA_distal_thickness_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               = numpy.std(WT_FGFR3_distal_thickness_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     hetero\_local\_post\_std = numpy.std(hetero\_FGFR3\_local\_
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    numpy.std(GpA_distal_thickness_list)
 bilayer
                                                                                                                                                                                                                                                                                             hetero_FGFR3_local_thickness_post_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               mutant_FGFR3_local_thickness_post_dimer_list)
                                                                                                                                                                                                                     hetero_FGFR3_local_thickness_pre_dimer_list)
                                                                                                                                                                                                                                                                                                                                                                                                         mutant_FGFR3_local_thickness_pre_dimer_list)
 the
 of
                                                                                                                                                                                                                                                                                                                                                                                                                                              mutant_local_post_avg = numpy.average
                                                                                                                                                                                                                                                              = numpy.average
                                                                                                                                                                                     = numpy.average(
 average value
                                                                                                                                                                                                                                                                                                                                                                         = numpy.average
 the
                                                                                                                                                                                                                                                        hetero_local_post_avg
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   mutant_local_post_std
                                                                                                                                                                                  hetero_local_pre_avg
                                                                                                                                                                                                                                                                                                                                                                mutant_local_pre_avg
                                                                                                                 II
 find
                                                                                                       WT_local_post_avg
                                                                                                                                            hetero_distal_avg
                                                                                                                                                                                                                                                                                                                               mutant_distal_avg
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                                                                        WT_local_pre_avg
 to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   WT_distal_std
                                   WT_distal_avg
     numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             444
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```
outfile.write('GpA' + str(GpA_distal_avg) + '' + str(GpA_distal_std)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                str (
                                                                                                                                                                                                                        local_post_avg | local_post_std
                                                                                                                                                                                                                                                                                                                                                  + ' + str(GpA_local_pre_avg) + ' ' + str(GpA_local_pre_std) str(GpA_local_post_avg) + ' ' + str(GpA_local_post_std)+'\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    str (
                                                                                                                           outfile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         + str(mutant_local_pre_avg) + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ' + str(mutant_local_post_avg)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          hetero_local_pre_std) + ' ' + str(hetero_local_post_avg)
                                                                                                                                                                                                                                                                                                                                                                                                                                                   str(
numpy.std(GpA_local_thickness_post_dimer_list)
                                                                                                                                                                                          | distal_avg | distal_std
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             + str(hetero_local_pre_avg) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          + str(mutant_distal_avg) + '
                                                                                                                              ล
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           outfile.write('hetero-FGFR3' + str(hetero_distal_avg)
                                                                                                                           dimer_batch_analysis_symlink/bilayer_histogram.out','w')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             WT_local_pre_std) + ' ' + str(WT_local_post_avg)
                                                                                                                                                                                                                                                                                                                                                                                                                                                 outfile.write('WT-FGFR3 ' + str(WT_distal_avg) + '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  +
                                                           gnuplot histogram plotting
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     simple_moving_average_polar_theta(folder_name, window_size):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             WT_distal_std) + ' ' + str(WT_local_pre_avg)
                                                                                            with open('/sansom/sc2/bioc1009/Documents/FGFR3_work/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           row for mutant FGFR3 results:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #next row for hetero FGFR3 results:
                                                                                                                                                                                     outfile.write('#format: category
                                                                                                                                                                                                                       local_pre_avg | local_pre_std
                                                                                                                                                                                                                                                 bilayer thicknesses in A) \setminus n')
                                                                                                                                                                                                                                                                                                                                                                                                               #next row for WT FGFR3 results:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         hetero_local_post_std)+'\n')
                                                                                                                                                                                                                                                                                      #next row is for GpA results:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          hetero_distal_std) + '''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     mutant_distal_std) + ' '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          WT\_local\_post\_std)+' \setminus n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       outfile.write('mut-FGFR3'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      mutant_local_post_std))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         mutant_local_pre_std)
                                                           #write the results to file for
                                                                                                                                                             format:
                                                                                                                                                           #row for file
   II
GpA_local_post_std
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1617 | def
                                      1603
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average now and also
                                                                                                                                                                                                                      categorized
                                                                                                                                                                                                                                                                                                                       window_size represents the size of the sliding window used to smooth the data
                                                                                                       average (SMA) for the FGFR3 polar theta tracking data (already present in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      a file in each
                                                                       simple moving
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  I don't filter
                                                                                                                                                                                                                                                                                                                                                                                                                                  which frame the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      on polar theta
                                                                                                                                               file) by taking advantage of the numpy.convolve method. My objective is
                                                                                                                                                                             the data so that I can more easily define polar theta boundaries
                                     a 'spike filter' to remove short-lived high-amplitude excursions
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              formation
                                                                                                                                                                                                                                                     all replicate trajectories
                                                                                                                                                                                                                   which primary and secondary dimer interface frames can be
                                                                  interfaces. Oct. 27/ 2010: This function will calculate the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      theta tracking values in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 actually forms (<7A separation between helices)--if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              the loop when the dimer
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    this then I could classify monomeric configurations based
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              comment lines
                                                                                                                                                                                                                                                                                                                                                                                                                                grabbing polar theta values I need to figure out in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          with open('closest_contacts_full.out','r') as contact_file:
'''Oct. 28/2010: The function is actually doing a weighted
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          dimer_start_frame = frame_number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  closest_contact = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frame_number = int(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          polar_theta_file_name = 'polar_interface_theta.out
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              if '#' in line: continue #skip
                                                                                                                                                                                                                                                        across
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      closest_contact < 6.0:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    already calculated the polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #leave
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                identified
                                                                                                                                                                                                                                                   overall interface analysis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         simulation directory:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              for line in contact_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        I don't want that:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           replicate
                                                                                                                                                                               smooth
                                                                                                                                                                                                                   around
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      dimer
                                                                                                                                                                                                                                                                                                                                                                                                                                  #Before
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #I have
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1633
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index])) > 1.0 and abs(polar_theta - numpy.average(theta_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           a way to ignore/attenuate large/short-lived polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     following averaged 20 data points, replace it by the average
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           of the 10 preceding points; note that it has to satisfy both
                                                                                                                                                                                                                                                                                                                                         if frame_number >= dimer_start_frame: #if the dimer has formed
                                                                                                                                                                                                                                                                                                                                                                                then include the polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  value is different by spike_threshold from the preceding
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        [index: index + 20])) > 1.0: #if the current polar theta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   abs(polar_theta - numpy.average(theta_list[index - 20
                                                                                                         theta_list = [] #initialize a list for storing polar theta values
                                                                      #initialize a list for storing the frame numbers
                                                                                                                                                                                                                              lines
                                                                                                                                                                                                                                                                                                                                                                                                                                                        frame_list.append(frame_number)
                                                                                                                                                                                                                        if '#' in line: continue #skip comment
                                                                                                                                                                                                                                                                                                                                                                                based on the closest contacts file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               theta_list.append(polar_theta)
                                                                                                                                               with open(polar_theta_file_name, 'r') as input_file:
                                                                                                                                                                                                                                                                                                     polar_theta = float(line.split()[1])
                                                                                                                                                                                                                                                              frame_number = int(line.split()[0])
= 'smooth_polar_theta.out'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          index = theta_list.index(polar_theta)
                                                                                                                                                                                                                                                                                                                                                                                                                    theta information:
                                                                                                                                                                                        for line in input_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    polar_theta in theta_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #this is tricky, but I'd like
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             if index > 20.0:
smooth_theta_file_name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 theta excursions:
                                                                     frame_list = []
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       counter = 0
                                              1637
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     447^{\overset{679}{1}}
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-over-list-slices/1339575#1339575 and the numpy.convolve online documentation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             We
                                                                                                                                                                                                                                          #numpy convolve trick (see http://stackoverflow.com/questions/1335392/iteration
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        smooth_theta_array = numpy.convolve(numpy.array(adjusted_coefficients), numpy
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 coefficient\_list = [coefficient for coefficient in range(1, window\_size + 1)]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            0
                                                                                              1
a real/long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      can leave the list of coefficients as increasing because the last value
                                                                                        = numpy.average(theta_list[index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      get reversed before convolution
                                                                                                                                                                                                                                                                                                        slid
                                                                                                                                                                                                                                                                                                        flipped and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            for unit_value, coefficient in zip(sliding_array, coefficient_list):
   conditions because I don't want to attenuate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              adjusted_coefficients.append(unit_value*coefficient)
                                                                                                                                                                                                                                                                                                                                  arguments):
                                                                                                                                                                                                                                                                                                                                                                                                entry)
                                                                                                                                                                                                                                                                                                                                                                                                                                                       denominator = (window_size * (window_size + 1.0)) / 2.0
                               between interfaces
                                                                                                                                                                                                                                                                                                      the second array argument
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 sliding_array = numpy.ones(window_size)/denominator
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   array(theta_list))[window_size-1:-window_size+1]
                                                                                                                                                                                                                                                                                                                                                                                               wikipedia
                                                                                                                                                                                                                                                                                                                                   default
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               average
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      adjusted_coefficients = [] #the list will
                                                                                      theta_list[index]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           print numpy.array(adjusted_coefficients)
                                                                                                                                                                                                                                                                                                                                                                                             weighted moving average (see WMA
                                                           counter += 1.0
                                                                                                                                                                                                                                                                                                                                  convolution with the
                                                                                                                        10: index])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              coefficients for weighted
                               lived transition
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   becomes the first value:
                                                                                                                                                                                                                                                                                                        how
                                                                                                                                                                                                                                                                                                        of
                                                                                                                                                                                                                                                                                                      explanation
                                                                                                                                                                                                                                                                                                                                   for
                                                                                                                                                        counter
                                                                                                                                                                                                                                                                                                                                   first
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               list of
                                                                                                                                                                                                                                                                                                                                                                                                   ಡ
                                                                                                                                                 print
                                                                                                                                                                                                                                                                                                                                                                                               #for
                                                                                                                                                                                                                                                                                                                                                                                                      1665
                                                                                                 1659
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                                                                                                                                                                                                                                                                                                                                                                                                                             448^{^{2}}
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be shorter and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     one of
                                                                                                                                                                                            of helix 1/n'
                                                                                                                                                              theta value
                                                                                                                                                                                                                                                                                                                                                                                                                                              #print str(folder_name), 'frame_number: ', str(frame_number),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         additionally
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        def interface_filtered_merged_top_five_FGFR3(primary_count_list,secondary_count_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         merges
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   interface. An
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             storing the frame numbers that correspond to primary
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ', str(smooth_data_point)
                                                                ', len(smooth_theta_array)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    filter all data based on weighted-average and spike-filtered polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'top five' contact data for wild-type, mutant, and hetero- dimers
                                                                                                                                                       polar
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    values available in the file 'smooth_polar_theta.out'. Parses and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                of 'main' in the head
                                                                                                                                                                                          2 COM position in rmsd-fixed reference frame
                                                                                                                                                                                                                                                                                     usually
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      '''Oct. 28/2010: Modifying the merged top 5 contacts function to
                                                                                                                                                                                                                                                                                                                                                    + str(
                                                                                                                                                                                                                                                        for frame_number, smooth_data_point in zip(frame_list,
                                                                                                                                                            smoothened
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              three lists: primary, secondary, or 'other' polar theta
                                                                                                                                                                                                                                                                                      smooth_theta_array): #the smoothened list will
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        other_count_list, folder_name, universe_object, skip_frames):
                               ', len(theta_list)
                                                                                                                                output_file:
                                                                                                                                                                                                                                                                                                                                                  output_file.write(str(frame_number) +
                                                                                                                                                           output_file.write('#format: frame number |
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              windowed polar theta value:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                additional function must be called outside
                                                                                                                                                                                                                                                                                                                                                                                  smooth_data_point) + ' \setminus n'
                                                                                                                                                                                                                                                                                                                                                                                                                  #monitor progress on prompt:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            parse the overall data in these lists.""
                                                            smooth_theta_array length:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        secondary, and 'other' dimer interfaces:
                                                                                                                             open(smooth_theta_file_name, 'w') as
                                    length:
                                  theta_list
         purposes:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #intialize lists for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         secondary_frame_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        primary_frame_list
                                    1a:
                                                                  1b:
testing
                                    'check
                                                               print 'check
                                 print
                                                                                                                                with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   1689
                                            1679
                                                                                                                                                                                                                                                                                                                                                                                                                                                 <sup>58</sup>
449
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                                                                                                        1681
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that interface
                                                                              want:
                                                                                                                                                                                                                                                                                                                                          ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                        current_index = frame_theta_list.index((frame_number,corrected_theta))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         current
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 this window that satisfy interface
                                                                                                                                                                                                                                                                                                                                     #Oct. 29/2010: adjusting the filtering to only allow frame classification in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        current_window: #check
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   -1.5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      conditions
                                                                                                                                                                                                                                                                                     each
                                                                           Н
                                                    just parse the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               satisfy interface requirements then this frame
                                                                          directory to pull out the values
                                                                                                                                                                                                                                                           corrected_theta))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      current_index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   corrected_theta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      points in the
                                                                                                                           ,'r') as corrected_theta_input_file
                                                                                                                                                                                                                                                                                    empirically
                                                                                                                                                                                                                                                                                                                                                             dimer interface if 50% of the subsequent points also fall into
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     -1.5:
                                                                                                                                                                              if '#' in line: continue #skip comment lines
                                                                                                    frame_theta_list = [] #store (frame number, corrected theta)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ..
                                                 I can
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     = frame_theta_list[current_index
                                                                                                                                                                                                                                corrected_theta = float(line.split()[1])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        V
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      the
                                                                                                                                                                                                                                                                                                                                                                                                                 for (frame_number, corrected_theta) in frame_theta_list:
                                                                                                                                                                                                                                                                                    I've determined
                                                                                                                                                                                                                                                        frame_theta_list.append((frame_number,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    corrected_theta > -3.0 and corrected_theta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       for (frame_number, corrected_theta) in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   corrected_theta > -3.0 and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            successful_test += 1
                                                 script handles the directory switching so
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        οĮ
                                                                                                                                                                                                        int(line.split()[0])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     20%
                                                                                                                                                       for line in corrected_theta_input_file:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               are satisfied:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     appended to interface list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    successful_test >= 5: #if
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   frames in
                                                                          smooth_polar_theta.out' file in each
                                                                                                                                                                                                                                                                                    the cutoffs
                                                                                                                              with open('smooth_polar_theta.out'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                primary interface
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  of
                                                                                                                                                                                                         frame_number =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         requirement
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            0
                                                                                                                                                                                                                                                                                     using
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              successful_test
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    current_window
                                                                                                                                                                                                                                                                                    data
                                                                                                                                                                                                                                                                                                              dimer interface:
   II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Ţ.
other_frame_list
                                                                                                                                                                                                                                                                                    #now filter the
                                                  #the head
                                1695
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1713
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                                                                                                                                                                                                                                                                                                                                                                 450
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FGFR3
                                                                                                                                                                                                                                                                                                                                                                                                                           correspond to which interfaces for the trajectory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       = universe_object.selectAtoms(helix_1_CA_selection)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 universe_object.selectAtoms(helix_2_CA_selection)
                                                                                                                                                                                                                                          can be
                        conditions
                                                                                                                                                                                                                 current
                                                                                                     frames in this window that satisfy interface
                                                                                                                                                                                                                                                                                                                                                                                                                                                      already been
                                                                           current_window: #check
                                                                                                                                                          < 1.0:
                                                                                                                                                                                                                                                                                                                     fall
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 second
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             first
                                                                                                                                                                                                                                       window satisfy interface requirements then this frame
                                                                                                                                                                                                                                                                                                                     not
                                                                                                                                                                                                             successful_test >= 5: #if 50% of the points in the
                                                                                                                                                           corrected_theta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                34:66 )" #select
                                                                                                                                                                                                                                                                                                                    frames where the polar theta of helix 2 does
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #select
                         -1.0 and corrected_theta < 1.0: #if
                                                                                                                                                                                                                                                                                         secondary_frame_list.append(frame_number)
                                                                                                                                                                                                                                                                                                                                                                                                                                                    are about to parse with MDAnalysis, and these frames have
primary_frame_list.append(frame_number)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          CA and resid 1:33 )"
                                                                           for (frame_number, corrected_theta) in
                                                                                                                                                          corrected_theta > -1.0 and
                                                                                                                                                                                                                                                                                                                                                                       other_frame_list.append(frame_number)
                                                                                                                                                                                successful\_test += 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  in universe_object.trajectory[::skip_frames]:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                resid
                                                 secondary interface are satisfied:
                                                                                                                                                                                                                                                              appended to interface list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 and
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              #Select all CA atoms in each helix:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          helix_1_CA_selection = "( name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           filtered to be within 6 Angstroms
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                _
=
                                                                                                     the number of
                                                                                                                                                                                                                                                                                                                                                category:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   II
                         elif corrected_theta >
                                                                                                                                requirement
                                                                                                                                                                                                                                                                                                                                                                                                                           #so, now we know which frames
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                helix_2_CA_selection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       helix_1_CA_residues
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_2_CA_residues
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         FGFR3 monomer
                                                                                                                                                                                                                                                                                                                    else: #for any
                                                                                                                                                                                                                                                                                                                                                into either
                                                                                                                                                                                                              ijĮ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           monomer
                                                                                                                                                                                                                                                                                                                                                                                                                                                          We
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    for ts
    1715
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number
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  for
                                                                                                                                                                                                                                                                            the
                                                                                                                                                                                                                                                                                                                                                                                     residue.
                                                                                                                                                                                                                                                                                                                                 residue
 coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    and
                                                                                                                                                                                                                                                                              at
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           helix_1_merged_list = zip(helix_1_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            helix_2_merged_list = zip(helix_2_ordered_residue_names_numbers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              23rd CA in helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #go through all possible combinations of residue interactions
                                                                                                                                       residue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    the distance (magnitude
                                                                                                                                                                                                                                                                           (in tuples
                                                                                                                                                                                                                                                                                                                                                                                                                                                                     #now zip the residue names and numbers with their respective
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    residue name
                                                                                                                                                                                                                                                                                                                                                                                    helix_2_ordered_residue_names_numbers = [(residue.resname,
                                                                                                                                                                                                                                                                                                                             helix_1_ordered_residue_names_numbers = [(residue.resname,
                                                     = helix_1_CA_residues.coordinates()
                                                                               helix_2_CA_residues.coordinates()
                                                                                                                                       ordered
  ordered
                                                                                                                                                                                                                                                                           numbers
                                                                                                                                                                 for each helix:
                                                                                                                                                                                        helix_1_identifiers = list(helix_1_CA_residues)
                                                                                                                                                                                                                    list(helix_2_CA_residues)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #this would give the [x y z] coordinate of the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 #the zeroth element would be the corresponding
                                                                                                                                    generate a list of
  o f
                                                                                                                                                                                                                                                                                                                                                                                                              resid) for residue in helix_2_identifiers]
                                                                                                                                                                                                                                                                                                                                                           resid) for residue in helix_1_identifiers]
                                                                                                                                                                                                                                                                           #make a list of ordered residue names and
generate lists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    a list of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        print helix_1_merged_list[22][1]
                                                                                                                                                               (MDAnalysis format)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    the two helices and make
  #Use the atomselections to
                                                                                                                                       atomselections to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_2_CA_coordinates)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_1_CA_coordinates)
                                                                                    II
                                                     helix_1_CA_coordinates
                                                                               helix_2_CA_coordinates
                                                                                                                                                                                                                       II
                                                                                                                                                                                                                     helix_2_identifiers
                                                                                                                                                                 identifiers
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 coordinates
                            each helix:
                                                                                                                                                                                                                                                                                                       moment)
                                                                                                                                       #Use the
      1735
                                                                                                                                                                                                                                                                                                                                                                                       452
452
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     1753
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```
helices
                                                                                                                                    measured_distance = numpy.linalg.norm(numpy.subtract(
                                                                                                                                                                                                                                                                       by the 'third element,' which is
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 overall
                                                                                                                                                                                                                                                                                                                            element [2])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           contains
                                                                                                                                                                                      labelled_interhelix_distances.append([helix_1_CA,
                                                                                                                                                                                                                                                                                                                                                                                 between
  retains the residue names that
                                                                                                          for (helix_2_CA, coordinate_2) in helix_2_merged_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                              #append the five closest contacts from this given frame to an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    distance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      primary_count_list.append(element) #still
                                                                                                                                                                                                                                                                                                                           = lambda element:
                                                                                                                                                                                                                                                                                                                                                                                 contacts
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   str(ts.frame)
                                                                               for (helix_1_CA, coordinate_1) in helix_1_merged_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        secondary_count_list.append(element)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 residue 1 and 2 names along with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for element in labelled_interhelix_distances[0:5]:
                                                                                                                                                                                                                   helix_2_CA, measured_distance])
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            categories
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             other_count_list.append(element)
                                                                                                                                                             coordinate_1, coordinate_2))
                                                                                                                                                                                                                                                                                                                                                                                 closest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            in secondary_frame_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              if ts.frame in primary_frame_list:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ' -- frame: ' +
                                                                                                                                                                                                                                                                                                                                                                                 οĘ
                                                                                                                                                                                                                                                                                                                            labelled_interhelix_distances.sort(key
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           based on polar theta interface
                                                                                                                                                                                                                                                                        the list of labelled distances
                                                                                                                                                                                                                                                                                                  the distance between CA particles
                                                                                                                                                                                                                                                                                                                                                                                 list
particles which
                            correspond to the interaction
                                                                                                                                                                                                                                                                                                                                                                                #so, now there is an ascending
                                                     labelled_interhelix_distances
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                          given frame
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ts.frame
 #between the CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  else:
                                                                                                                                                                                                                                                                                                                                                                                                           for this
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            list
                                                                                                                                                                                                                                                                          #sort
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  print
      1755
                                                                                       1757
                                                                                                                                                                                                                                                                                                                                                                                   <sup>59</sup><sub>2</sub>
453
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dimer_batch_analysis_symlink/test_case_top5/%s_merged_topfive.out'
   function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         helix_1_partner_list.append(helix_1_partner) #the involved
                                                                                                                                                                                                                  '''Oct. 28: Modifying this function to work for polar-theta-filtered data.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 duplicates) found in close contacts from the respective helices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      for (helix_1_partner, helix_2_partner, separation) in input_list:
                                                                                                                                                                                                                                                        οĘ
                                                                       primary, secondary, and other dimer interfaces
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                #make separate lists with each of the residue names (including
                                                                                                                                                                                                                                                    each
                                                                                                                                        def interface_filtered_parse_overall_FGFR3_top_five_data(primary_count_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               οĘ
the contact count lists will be parsed by another
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             most
                                                                                                                                                                                                                                                  Perform final analysis on merged 'top five contacts' data for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                file_path = '/sansom/sc2/bioc1009/Documents/FGFR3_work/
                                     for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         condition, num_pairs): #does
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        open(str(file_path),'w')
                                     data
                                                                                                                                                                                                                                                                                    three FGFR3 *dimer interfaces* and print to file.'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         residue from helix 1 gets appended
                                     the final
                                                                                                                                                                                                                                                                                                                                                            #determine number of top five contacts in total:
                                                                                                                                                                                                                                                                                                                                                                                                                                 num_secondary_pairs = len(secondary_count_list)
                                                                                                                                                                                                                                                                                                                                                                                             num_primary_pairs = len(primary_count_list)
                                     main to get
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   num_other_pairs = len(other_count_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           pairs
                                                                                                                                                                                secondary_count_list, other_count_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        merged_top_five_outfile =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       def parser_printer(input_list,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_1_partner_list =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           a given list of contact
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            print len(input_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              helix_2_partner_list
                                     οĘ
                                     called outside
                                                                      contacts' for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      condition
   #each of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         <sup>58</sup>454
                                                                                                                   1779
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#raw
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    parser_printer(secondary_count_list, 'secondary_interface', num_secondary_pairs
                                                                                                                                                                                                                                                                                                                                                                                                                                                         residue1_frequency) + ' ' + str(residue2_name[0]).strip("'")
                                                                                                                                                                                                                                                                                                                                                                          merged_top_five_outfile.write(str(residue1_name[0]).strip("'")
                                                                                                                            #
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          merged data for each
                                                                                                                          = float(residue1_count)/float(num_pairs)
                                                                                                                                                                                                            = float(residue2_count)/float(num_pairs)
residue2_count = helix_2_partner_list.count(residue2_name)
                                                                                                                                                                                                                                                                                                                                  residue numbers to match those used in FGFR3 literature
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          parser_printer(primary_count_list, 'primary_interface', num_primary_pairs)
                                                                                                                                                                                                                                                                                          #print out results that are simultaneously corrected for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   + ' ' +str(residue2_name[1]+(366-33)) + ' ' + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      parser_printer(other_count_list, 'other_interface', num_other_pairs)
                                                                                                                                                                                                                                                                                                                                                                                                              + ' +str(residue1_name[1]+366) + ' + str(
                                                                                                                                                                  because we want the 'normalized frequency'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #now call the function to parse and print three files of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             residue2_frequency) + ' \setminus n')
                                          residue counts for helix2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       merged_top_five_outfile.close()
                                                                                                                          residue1_frequency
                                                                                                                                                                                                        residue2_frequency
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  FGFR3 dimer interface:
        1813
                                                                                                                                    1815
                                                                                                                                                                                                                                                             1817
                                                                                                                                                                                                                                                                                                                                                                                       1819
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1827
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      1825
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           456^{\frac{7}{28}}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              1823
```

isting D.18: This module (analyze_FGFR3_monomer_simulations.py) serves a simi-D.19 on page 460

```
paths
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               tuple
                                                                    files organized in a directory with symlinks to the original production-length
           carried
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               2) in the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               a list of tuples with pairs of pdb (index 0) and xtc (index 1)
                                                                                                                                                                                                                                                                                                                                                                                        = ['mutant_monomer_replicate_1','mutant_monomer_replicate_2'
        ٦.
ا
    of the ten FGFR3 monomer trajectories. Analysis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          for a given simulation folder. The name of the folder is (index
                                                                                                                                                                                                                                                                                             monomer_symlink_directory = '/sansom/sc2/bioc1009/Documents/FGFR3_work,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 def list_data_paths(monomer_symlink_directory,folder_name_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    wildtype_monomer_replicate_2','wildtype_monomer_replicate_3'
                                                                                                                                                                                                                                                                                                                                                                                                                                                     'mutant_monomer_replicate_5', 'wildtype_monomer_replicate_1'
                                                                                                                                                                                                                                                                                                                                                                                                                     mutant_monomer_replicate_3','mutant_monomer_replicate_4'
                                                                                                                                                                                                                                                                                                                             monomer_batch_analysis_symlink/'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           list_of_pdb_file_paths=[]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     wildtype_monomer_replicate_4'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'wildtype_monomer_replicate_5']
                                                                                                                                                                                                  monomer_geometric_tools
        analysis
                                                                                                 folders.'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               '''Returns
                                                                                                                                                                MDAnalysis
                                                                                                                                                                                                                                                                                                                                                                                          folder_name_list
      specific
                                                                        parsing
                                                                                                      data
''' For
                                                                                                                                                                   import
                                                                                                                                                                                                   import
                                                                                                                                                                                                                                  import
                                                                                                                                                                                                        n
                                                                                                                                                                                                                                                                                                                                                                                                                                                               11
                                                                                                                                         က
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    17
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```

```
appropriate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            functions '''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      gnuplot format:
                                                                                                                                                                               = zip(list_of_pdb_file_paths,list_of_xtc_file_paths
                         differently
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         def main(monomer_symlink_directory,folder_name_list,skip_frames,geo_z_output_file
                                                                            folder
                                                                                                                              folder
                                                                                                                                                                                                                                                                                                                                                                                                                                     universe_list.append([MDAnalysis.Universe(pdb_file,xtc_file),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 them to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     os.chdir(monomer_symlink_directory + folder_name) #move the
                                                                                                                                                                                                                                                                                   create_universe_selections(monomer_symlink_directory,folder_name_list):
                                                                                                                                +
                                                                              +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            various
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 functions
                           for
                                                  not)
                                                                                                                              list_of_xtc_file_paths.append(monomer_symlink_directory
                                                                            list_of_pdb_file_paths.append(monomer_symlink_directory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     create_universe_selections(
                                                                                                                                                                                                                                                                                                            system
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        be in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  comment out functions when you are not needing
                           changed
                                                      OL
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          calling
                                                   simplified and centered
                                                                                                                                                                                                                                                                                                                                                                                     pdb_file, xtc_file, folder_name in list_data_paths(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       should
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               directory before executing data processing
                                                                                                                                                                                                                                                                                                             ಡ
                           can be
                                                                                                                                                                                                                                                                                                            atoms in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            βŊ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        functions; output
                                                                                                                                                                                                                                                                                                                                                                                                              monomer_symlink_directory, folder_name_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              monomer_symlink_directory,folder_name_list):
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            directories
                                                                                                                                                     'prod.xtc') #centered_CA_POPC.xtc
                                                                                                    + 'prod.gro') #centered_CA_POPC.pdb
                              names
                                                                                                                                                                                                                                                                                                          (all
                         folder_name_list: #files
                                                                                                                                                                                                                                                                                                          nested list of [universe
                                                                                                                                                                                                                                                                                                                                   objects, folder_name]'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            symlink
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for universe_object, folder_name in
                                                  (i.e.,
                                                                                                                                                                                                                                return combined_path_pair_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       data processing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            data to files in
                                                   trajectories
list_of_xtc_file_paths=[]
                                                                                                                                                                               combined_path_pair_list
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 {	t folder\_name]})
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           universe_list
                                                                                                                                                                                                       folder_name_list)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    helix_tilt_output_file):
                                                                                                                                                                                                                                                                                                                                                           universe_list=[]
                             in
                                                                                                                                                                                                                                                                                                                                    MDAnalysis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        #call
                                                                                                                                                                                                                                                                                                          '''Creates
                                                                                                                                                                                                                                                                                    def
                                19
                                                                                                                                                                                                                                                                                       25
                                                                                                                                                                                                                                                                                                                                                              5
458
                                                                                                                                                                                                                                      23
                                                                                                                                                                                                                                                                                                                                                                                                                                              29
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      37
                                                                                                                                   ^{21}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       31
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  33
```

```
geo_z_output_file='Z_tracking_skip10_no_center.out', helix_tilt_output_file='
                                                                                                                  universe_object, skip_frames, output_file=helix_tilt_output_file)
monomer_geometric_tools.geo_z_tracker(folder_name, universe_object,
                                                                         #monomer_geometric_tools.helix_tilt_vs_bilayer_normal(folder_name,
                                                                                                                                                                                                                                                                          main(monomer_symlink_directory, folder_name_list, skip_frames=10,
                                   skip_frames, output_file=geo_z_output_file)
                                                                                                                                                                                                                                                                                                                                                 tilt_test.out')
                                                                                                                                                                                                                                      __main__
                                                                                                                                                                                                                                        II
                                                                                                                                                                                                                                        --name--
                                                                                                                                                                                                                                    if
                                                                                                                                                                                                                                            43
       39
                                                                                                                                                                 41
```

ions that can be called by the head script (analyze_FGFR3_monomer_simulations.py D.18 on page 457 to parse the FGFR3 monomer replicate MD trajectories

```
ಡ
                                                                                                                                                                                                                                                                                                                                                                                                  particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         for ts in universe_object.trajectory[::skip_frames]: #xtc trajectory
                                                                                                                                                                                                                            in
                                                                                                                                                                                                                                                                                                                                                                                                                                                a given frame, with frame intervals defined by skip_frames, to
                                                                                                                                                                                                                           particles
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        geo_z_tracking_file.write("#column 1: frame number || column
                                                                                                                                                                                                                                                                                                                                                                                                  '''Prints the Z-coordinate of the geometric center (only CA
                                                                                                                                                                                                                                                                                                                                                                           output_file):
                                                                                                                                                                                                                           CA
                                                                                                                                                                                                                            mass for
      motion
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               geometric center for CA-monomer \langle n^{"}\rangle
   analyzing the
                                                                                                                                                                                                                                                                                                                                                                           geo_z_tracker(folder_name, universe_object, skip_frames,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 geo_z_tracking_file = open(output_file ,'w')
                                                                                                                                                                                                                         geometric center of
                                                                                                                                                                                                                                                                                                                                                                                                                           given universe_object
                                                                                                                                                                                                                                                                                                 geometric_center_xyz = ca.centerOfGeometry()
                                                                                                                                                                                                 geometric_center_z_coordinate_CA(selection):
  MDAnalysis tools here for
                                                                                                                                                                                                                                                                        = selection.selectAtoms("name CA")
                        monomer peptide in an MD trajectory.'''
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       specified output_file)'''
                                                                                                                                                                                                                          '''Returns Z coordinate of the
                                                                                                                                                                                                                                                                                                                          geometric_center_xyz[2]
                                                                                                                                                                                                                                                                                                                                                                                                                              ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                           for
                                                                                                                                                                                                                                                                                                                                                                                                                          considered)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   position of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     object
                                                                                                                                                                                                                                                  selection.'''
'''Start building up
                                                                          MDAnalysis
                                                                                                                                                                                                                                                                                                                            return
                                                                                                  numpy
                                                                                                                          math
                                                                          import
                                                                                                   import
                                                                                                                           import
                             ಡ
                                                                                                                                                                                                     def
                                                                                                                                                                                                                                                                                                                                                                            def
                                                                                                                                                                                                                                                                                                                                                                                 15
                                                                                                                                                                                                                                                            460
                                                                                                                                                                                                                                                                                                                                13
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                19
                                                                                                                                                                                                                                                                                                                                                                                                                                                        17
```

```
def helix_tilt_vs_bilayer_normal(folder_name, universe_object, skip_frames, output_file
                                                                                                                                                                                                                                       to bilayer normal using
                                                                                                                                                                                                                                                                                                                                                                        tilt angle
each frame of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        substract
                                                                            \tt geometric\_center\_z\_coordinate\_CA(universe\_object))+' \backslash n')
                                                                                                                                                                                                                                                                                            normal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                = bilayer_phosphate_selection.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      bilayer_phosphate_selection = universe_object.selectAtoms("name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Jo
                                                                                                                                                                                                                                                                                           and bilayer
                                                                                                                                                                                                                                                                                                                                                                        helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                coordinates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       coordinates; so
                                                                                                                                                                                                                                                                                                                                                                                                 approach) \n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         bilayer_phosphate_selection
 center for
                                                                                                                                                                                                                                                                                                                                                                       1: frame # || column 2:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CA\_selection = universe\_object.selectAtoms("name <math>CA")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CA_selection.centerOfGeometry()
                                                 geo_z_tracking_file.write(str(ts.frame) +
                                                                                                     : ' + str(ts.frame)
                                                                                                                                                                                                                                       tilt of helix (CA backbone) relative
                                                                                                                                                                                                                                                                                           eigenvector)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   the
                                                                                                                                                                                                                                                                                                                                                                                                                          in universe_object.trajectory[::skip_frames]:
geometric
                                                                                                                                                                                                                                                                                                                                                                                                  degrees) relative to bilayer normal (eigenvector
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          CA_selection.coordinates()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                geometric center coordinate value from
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        centered
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          #when you call the numpy SVD function:
                                                                                                                                                                                                                                                                                                                                            open(output_file, 'w')
                          simulation (every nth
                                                                                                                                                                                                                                                                                           (first
 coordinate of CA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       a SVD on the
                                                                                                                                                                                                                                                                                                                                                                        helix_tilt_angle_file.write('#column
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           II
                                                                                                      print folder_name +
                                                                                                                                geo_z_tracking_file.close()
                                                                                                                                                                                                                                                                                            axis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           phosphate_geometric_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    II
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 phosphate_coordinates
                                                                                                                                                                                                                                                                                           SVD technique to define helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    centerOfGeometry()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CA_geometric_center
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #the idea is to do
                                                                                                                                                                                                                                                                                                                    third eigevector).''
                                                                                                                                                                                                                                                                                                                                               helix_tilt_angle_file =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          CA_coordinates
                                                                                                                                                                                                                                        the
                                                                                                                                                                                                                                                                   algebra
                                                                                                                                                                                                                                    '''Calculates
     21
                                                                                                             23
                                                                                                                                                                 25
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               41
                                                                                                                                                                                                                                             27
                                                                                                                                                                                                                                                                                                                                                                                                                                   31
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            37
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                39
                                                                                                                                                                                                                                                                                                                                                                          461
```

```
bilayer (phosphates
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          = numpy.linalg.svd(bilayer_phosphate_selection.coordinates()
                              in VMD,
                                                                                                                                                                      the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                then
                                                                                                                                                                                                                                                                                                                                            certain frames
                                                                                                                                                                                                                                                                                                      output.log")
uu, dd, vv = numpy.linalg.svd(CA_coordinates-CA_geometric_center)
                                                                                                                                                                      'direction' from
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  [coordinate * 7 for coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              extended vector,
                              helix_vector = vv[0] #this is the first eigenvector; checking
                                                                                                     that
                                                                                                                                                                                                                                                                                                                                                                                                             here
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     lower_helix_vector_coordinate, CA_geometric_center))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      upper_helix_vector_coordinate, CA_geometric_center))
                                                                                              #vv[1] and vv[2] are the other two eigenvectors; note
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             -7 for
                                                                                                                                                                                                                                                                                                                                                                                                         challenges
                                                                                                                                                                                                                                                                                                        #os.system("vmd -dispdev text -eofexit < input.tcl >
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             add the geometric center back for the given frame:
                                                                                                                                                                                                                                                                                                                                          render the vectors for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         #repeat the eigenvector calculation process for the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            -bilayer_phosphate_selection.centerOfGeometry()]
                                                                                                                                                                         ಡ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #lower_helix_vector_coordinate = [coordinate *
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            #for testing: multiply by -7 and +7 to make an
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    #lower_helix_vector_coordinate = map(sum, zip(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      #upper_helix_vector_coordinate = map(sum, zip(
                                                                                                                                                                       ន
                                                                                                                                                                                                    origin (0,0,0) for drawing the given vector
                                                                                                                                                                      an (x,y,z) coordinate that serves
                                                                                                                                                                                                                                                                                                                                                                                                              some
                                                                                                                                     these eigenvectors the result you get
                                                                    axis
                                                                                                                                                                                                                                                                                                                                                                                                                are
                                                                                                                                                                                                                                                                                                                                                                                                           but I think there
                                                                 it looks right along the helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  #upper_helix_vector_coordinate =
                                                                                                                                                                                                                                                                                                                                          could eventually set up to
                                                                                                                                                                                                                                                                                                                                                                                                        #automatically,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 helix_vector]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          aa, bb, cc
                                                                                                                                                                                                                                                                           #import os
                                                                                                                                                                    #back is
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str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             vectors depends on the dot
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #
      seems
                                                                                                      in
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     str(theta) + ' n')
                                                                                                                                                                                                                                                                                                                                                                                                                 (lower_bilayer_vector_coordinate) + '\n' + "upper bilayer coord:
                                                                                                       coordinate
                                                                                                                                                                                                                                                                                                                                                                                         =
                                                                                                                                                          coordinate
= cc[2] #check in VMD: yes, the third eigenvector
                                                                                                                                                                                                                                                                                                                                                                                           coord:
                        produce a reasonable vector up +Z axis for bilayer normal
                                                                                                                                                                                                                                   lower_bilayer_vector_coordinate,phosphate_geometric_center))
                                                                                                                                                                                                                                                                                    upper_bilayer_vector_coordinate,phosphate_geometric_center))
                                                                                                                                                                                                                                                                                                                                                                 "upper helix coord:
                                                                                                                                                                                                                                                                                                                                                                                       "lower bilayer
                                                                                                                                                                                                                                                                                                                                     " + str(
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             angle = math.acos(numpy.dot(helix_vector,bilayer_normal))
                                                                                                       for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        str(ts.frame)
                                                                                                                                                          for
                                                                                                                                                       _
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        +
                                                                                                                                                                                                                                                                                                                                        coord:
                                                                                                          <del>*</del>
                                                                                                                                                           *
                                                                                                                                                                                                           map(sum, zip(
                                                                                                                                                                                                                                                               map(sum, zip(
                                                                                                      [coordinate
                                                                                                                                                         [coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                         str(upper_bilayer_vector_coordinate) + '\n')
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               and bilayer normal
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        +
                                                                                                                                                                                                                                                                                                                                                               + 'n'
                                                                                                                                                                                                                                                                                                                                                                                      + 'n' +
                                                                                                                                                                                                                                                                                                                                       #helix_tilt_angle_file.write("lower helix
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     helix_tilt_angle_file.write(str(ts.frame)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 columns
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         frame:
                                                                                                       #lower_bilayer_vector_coordinate =
                                                                                                                                                           II
                                                                                                                                                                                                           #lower_bilayer_vector_coordinate =
                                                                             for the helix vector:
                                                                                                                                                                                                                                                                                                                                                                                        upper_helix_vector_coordinate)
                                                                                                                                                                                                                                                                                                                                                                  lower_helix_vector_coordinate)
                                                                                                                                                         #upper_bilayer_vector_coordinate
                                                                                                                                                                                                                                                             #upper_bilayer_vector_coordinate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               gnuplot-ready
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   = math.degrees(angle)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             #angle between helix axis
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    theta = 180-theta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       print str(folder_name) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       #convert to degrees
                                                                                                                                                                                bilayer_normal]
                                                                                                                                bilayer_normal]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               formatting for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          if theta > 90:
   bilayer_normal
                                                                            #testing, as
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       product:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   theta
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