

Modeling and analyzing secondary structure differences based on changes in the hinge region of the FXR nuclear receptor

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INTRODUCTION

The farnesoid X receptor (FXR) is a bile acid nuclear receptor found mostly in the liver and intestines. FXR is a promising drug target for the therapy of bile acid-related liver diseases⁴.

FXR includes three parts which can be modeled: a DNA binding domain (DBD), a ligand binding domain (LBD), and a connecting hinge. When activated by a ligand, a molecule that binds to a portion of the LBD, the protein undergoes a conformational, or shape, change which may cause the transcription and translation of specific proteins. [Figure 1; Figure 2]]

Mutations in the gene that encodes the FXR protein may participate in various diseases and understanding how variations to the protein structure affect the protein's motion may assist in the development of drug therapies. In many cases, any changes to a protein's structure may affect its ability to function properly.

In the study published by the Okafor Lab at Penn State, computer simulations of FXR showed that the LBD and the DBD had physical interactions only in the presence of the hinge⁵. Based on this, the main question for this study is: How does changing the hinge affect the overall protein's trajectories and secondary structure?

SEQUENCE OF RESIDUES (AMINO ACIDS) IN FXR

RIKQDELVCVGDGRASGYHYNALTCGCKGFFRRSITKNVAVYKCKNGGNCVMDMYMRR
KCQECRLRCKEMGLAECMYTGLLTIQCKSKRLRKNVQKQADQTVNESEGRDLRQ
VTSTTKSCRKELTEPDQQLLHFIMDSYNKQRMPEITNKILKEEFAENFLILTEMATN
HVQVLVEFTKLPFGQTLDEHQIALLKGSVAEAMFLRSAEIFNKKLPSGHSDLLEERIRNS
GISDEYITPMFSFYKSGELKMTQEEYALLTAIVLSPDRQYKIDREAVKLEPLLDVQLKLC
IHQENPQHFACLLGRLELTFNHHAAEMLSWRVNDHKFTPLLCIWDVQ

Residues removed/changed for each of the hinge "sections"

HINGE 1: MYTGLLTIQCKS
HINGE 2: KRLRKNV
HINGE 3: KQHADQTVNESEGRDLR
HINGE 4: QVTSTTKSCR
HINGE 5: Replaced the hinge with Glycines and S
NO HINGE: Removes the entire colored portion of amino acids

Figure 3: Sequences of amino acids in FXR; Identification of the hinge sections that are removed.

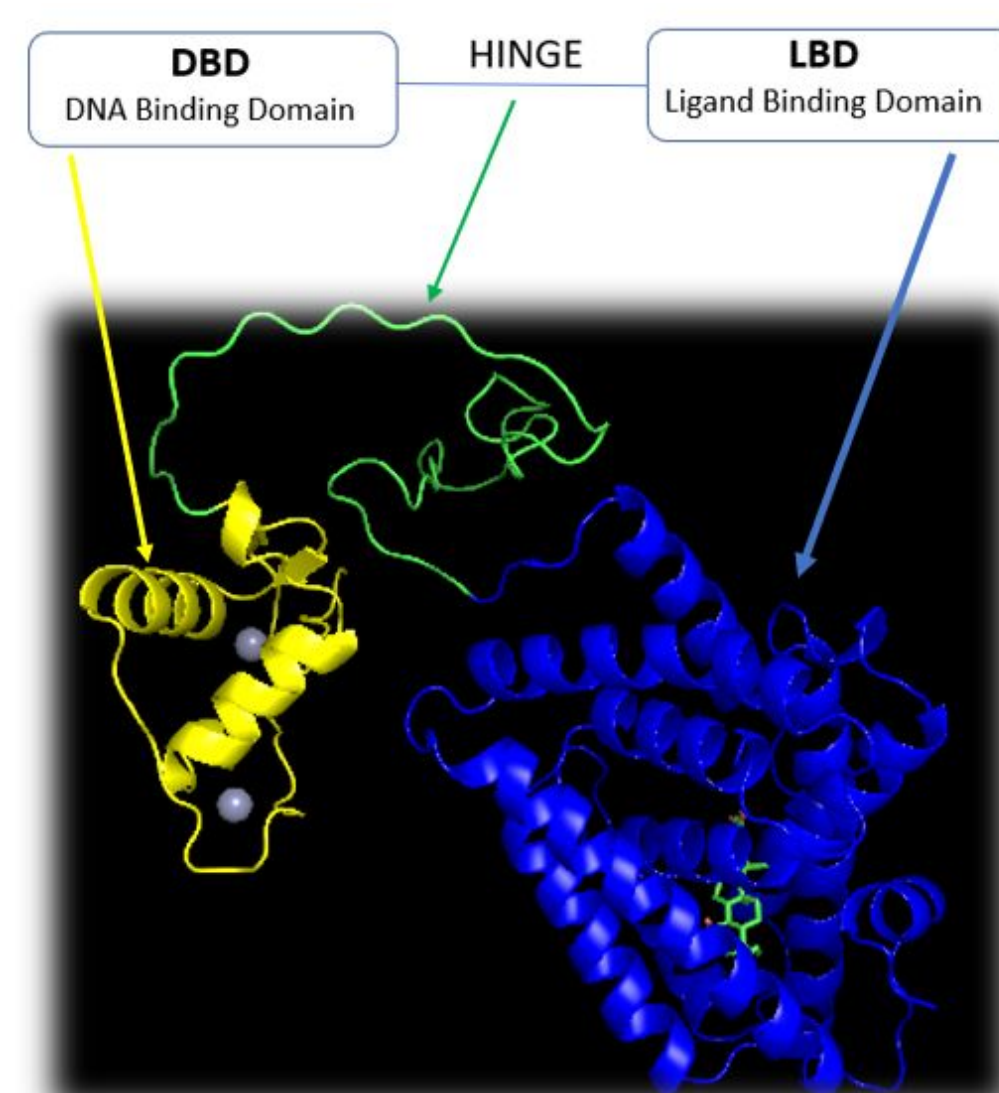


Figure 1: Molecule visualized in PyMOL

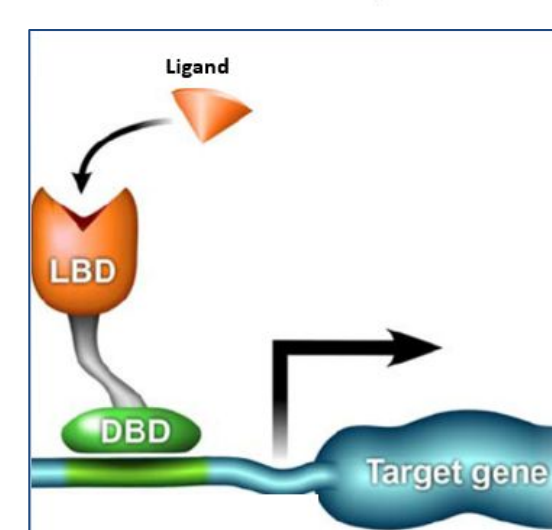
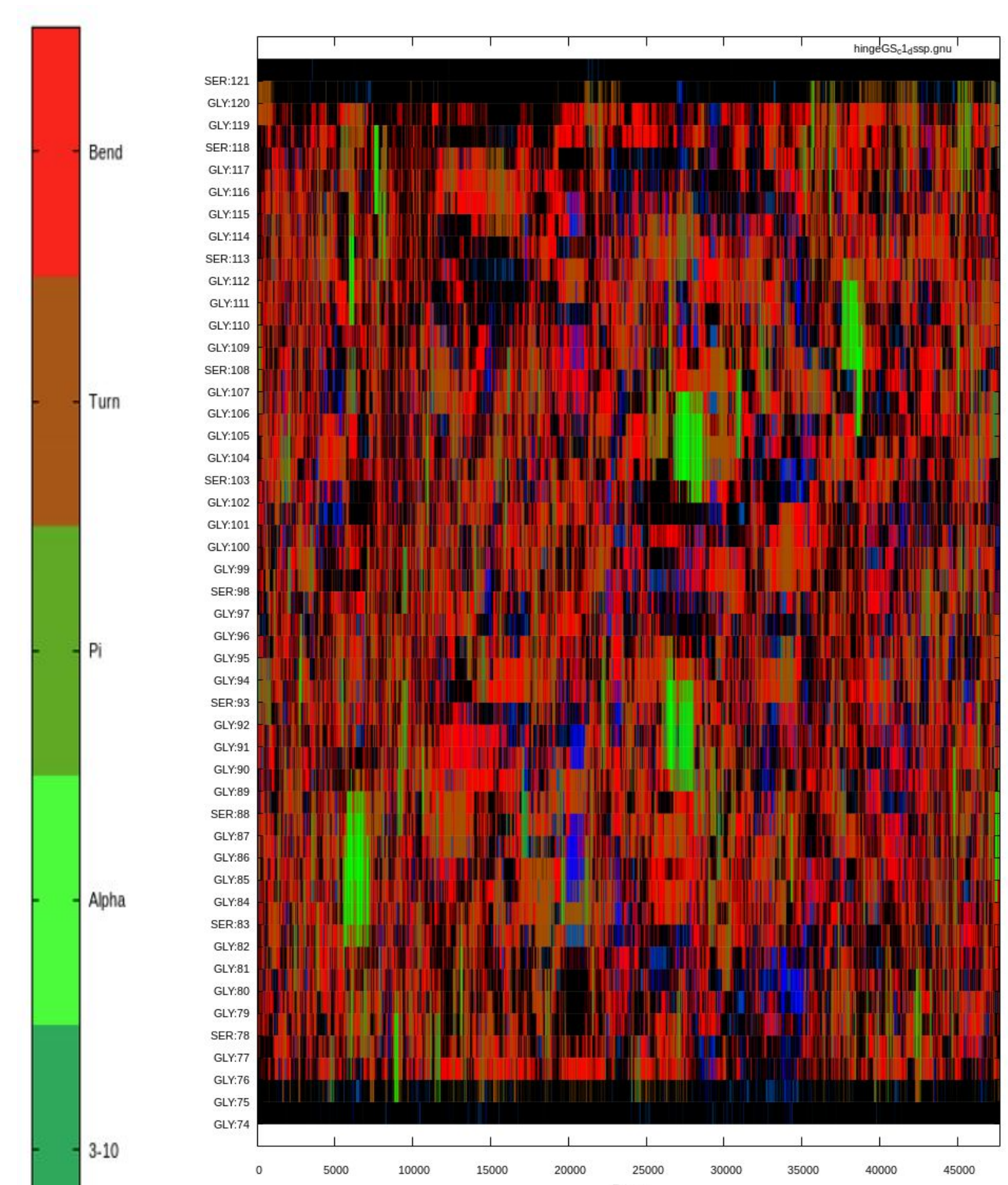


Figure 2: Nuclear receptor

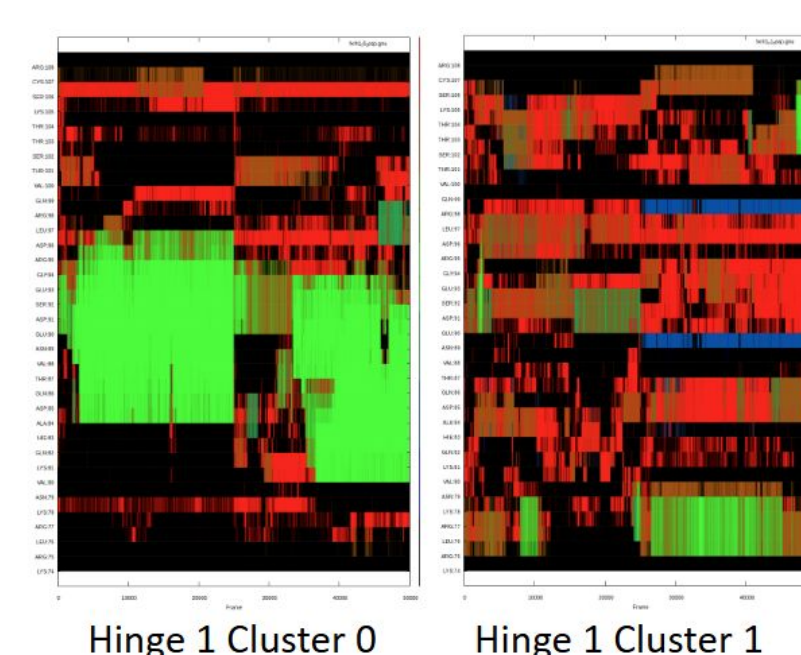
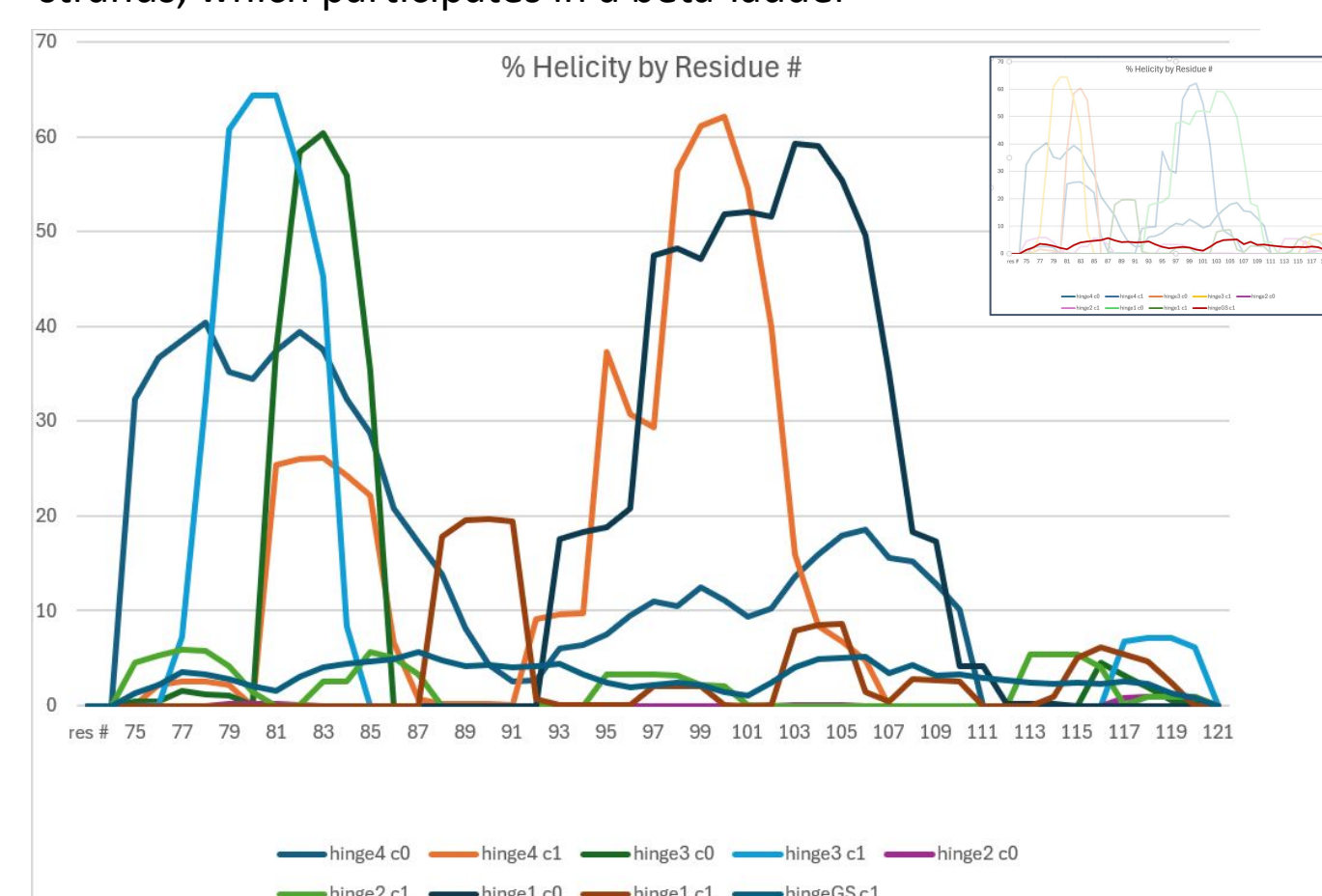
This study uses a computational modeling approach, including the use of accelerated Molecular Dynamics (aMD), to study the trajectories and secondary structures of the FXR with portions of the hinge removed.

RESULTS

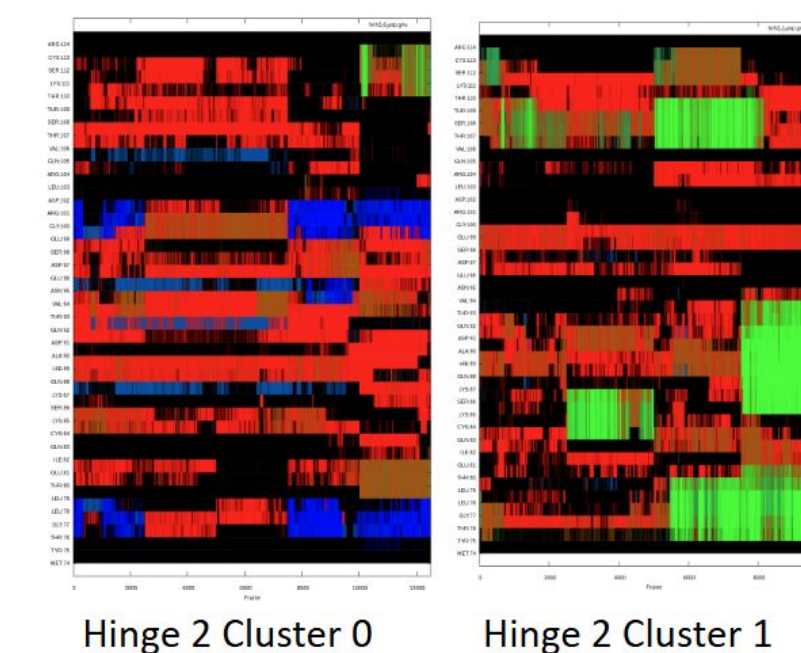


DSSP Graphs: Shows secondary structures present at each residue over the trajectories

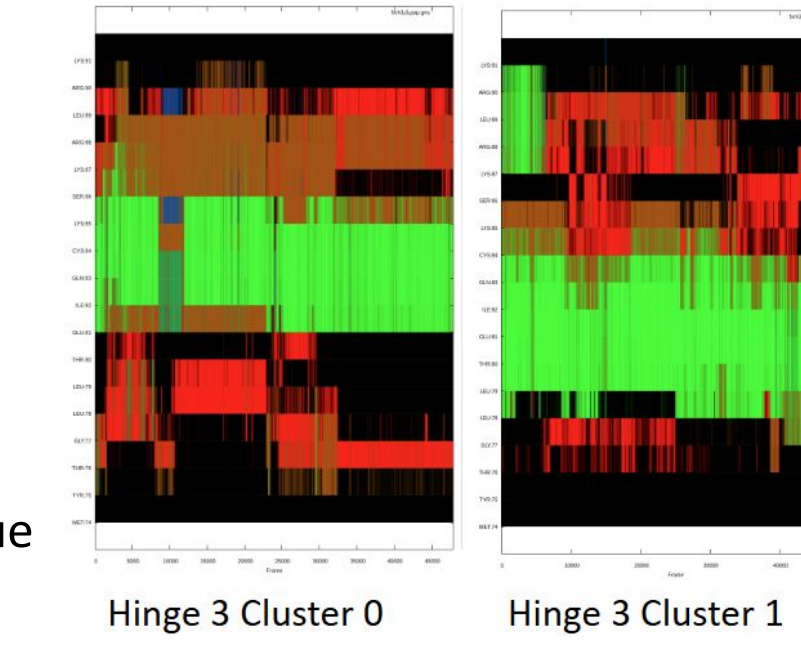
Figure 4 (Left): DSSP secondary structure colors for the DSSP data
Figure 5 (Above): DSSP data for Hinge GS: Primarily red shown, which indicates bends in the secondary structure according to figure 4; Green shows areas of alpha helices, bright blue shows areas of extended strands, which participates in a beta-ladder



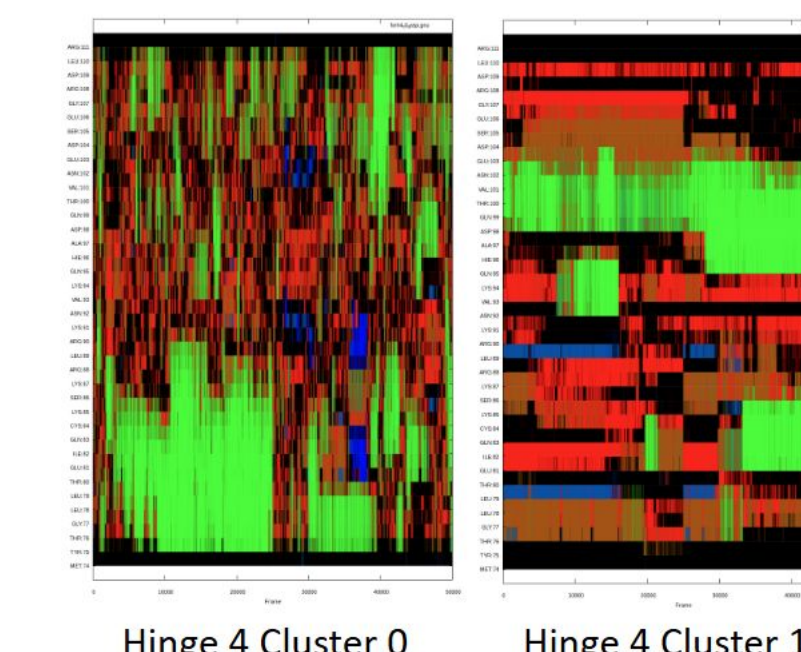
Hinge 1 Cluster 0 Hinge 1 Cluster 1



Hinge 2 Cluster 0 Hinge 2 Cluster 1



Hinge 3 Cluster 0 Hinge 3 Cluster 1

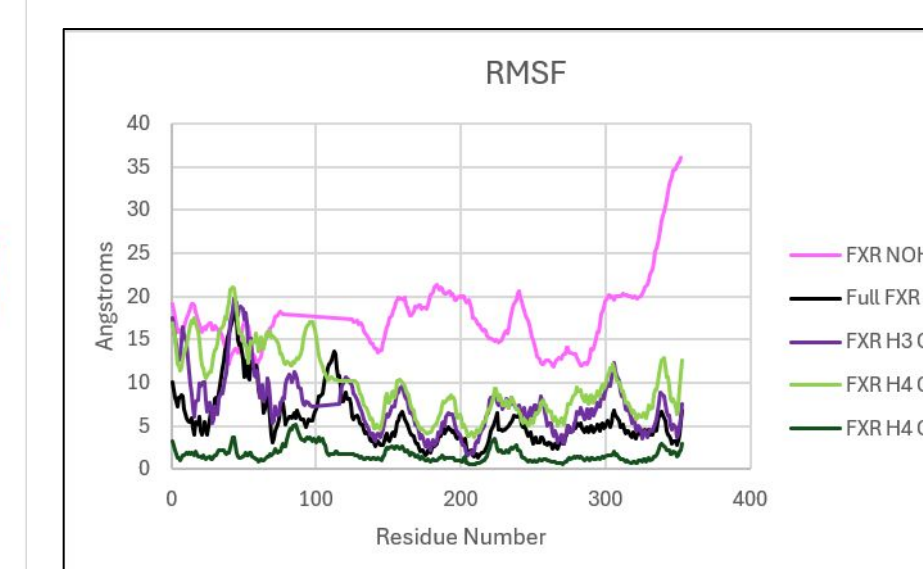
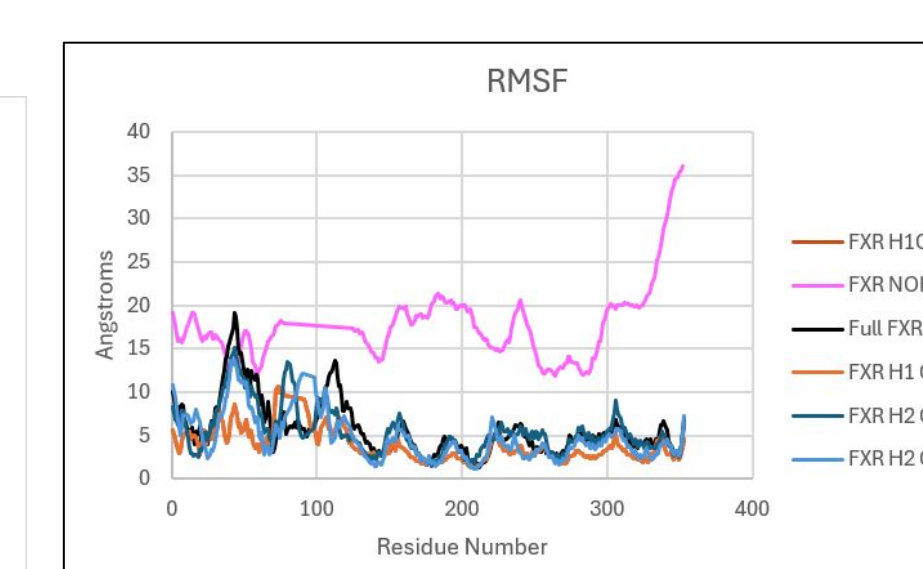
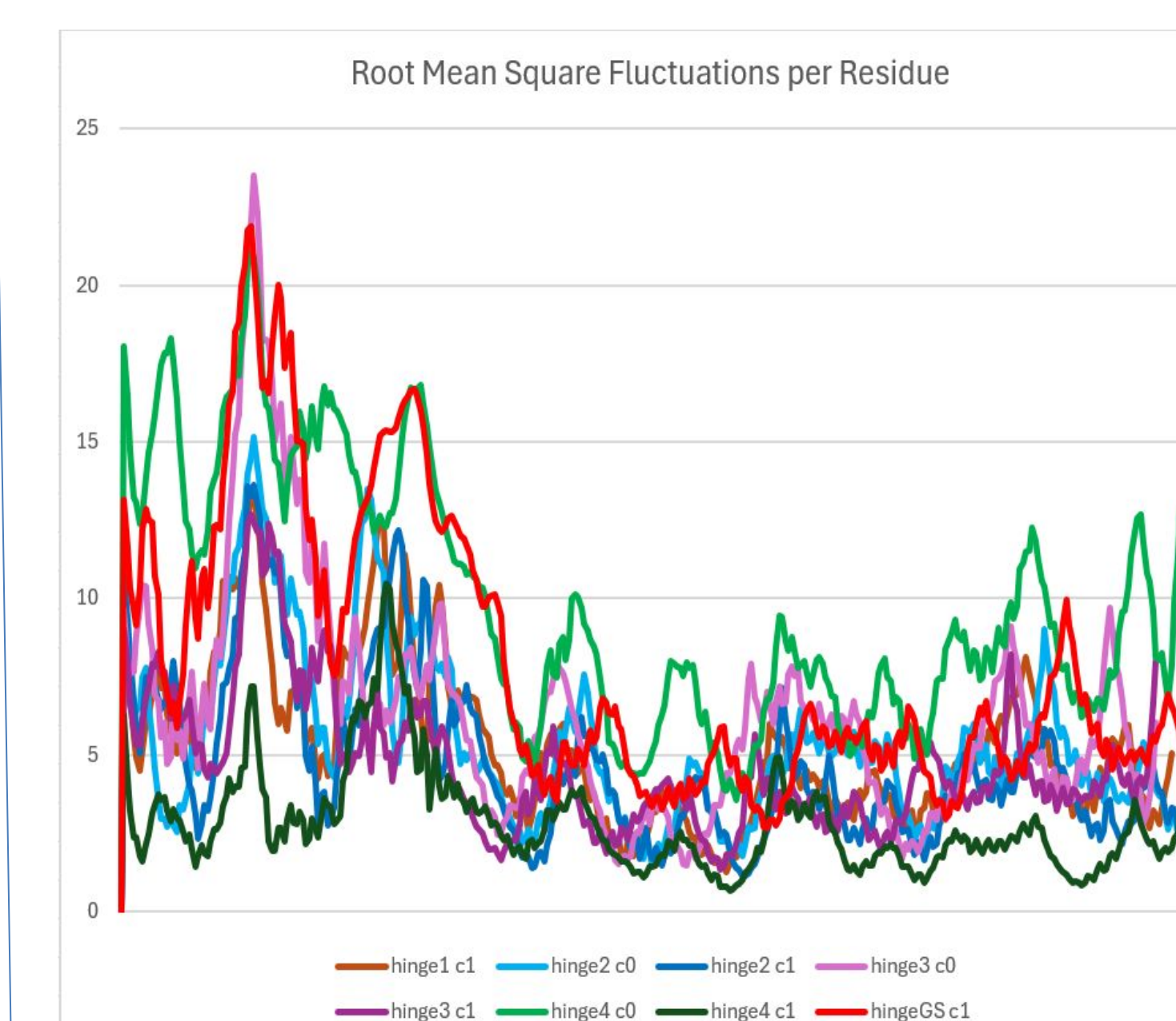


Hinge 4 Cluster 0 Hinge 4 Cluster 1

Figure 6 (Above): DSSP data for Hinges 1 - 4 both clusters

Figure 7 (Left): % Helicity by Residue # graph with comparative inset of the red HINGE5 line (HINGE 5 generally avoids helicity)

RESULTS



RMSD Graphs: Show the root-mean square deviation of the combined trajectory data. This compares general fluctuation of the whole system from the reference frame.

Figure 8 (above): RMSD comparisons across all simulated versions of the FXR protein

Figure 9 (top right): Compares the FXR protein to versions with no hinge, missing hinge 1 and missing hinge 2.

Figure 10 (top left): Compares the FXR protein to versions with no hinge, missing hinge 3 and missing hinge 4.

CONCLUSIONS

- Based on both of the RMSD and RMSF data, there is less stability of the protein (more fluctuations) when hinge residues are removed closer to the LBD than the DBD.
- For the RMSF data, the motion of the LBD is more conserved showing much more variability in the DBD and hinge portions of the FXR versions.
- When there is no hinge, there are significant fluctuations, making the molecule unstable
- DSSP shows bends as part of the secondary structure of all the hinge portions
- Hinges 1 and 2 show more extended strands, which participate in the Beta-ladder than the other hinge regions
- There is a lower percent helicity in hinge 2 versus hinge regions 1, 3, and 4
- Hinge 3 shows consistent helicity (60 - 70%) across clusters

METHODS

MODEL
Using PyMol, Modeller, Linux

MINIMIZE COMPLEX
Using Amber, Python, Linux

RUN ACCELERATED
MOLECULAR DYNAMICS
Using Linux, Python

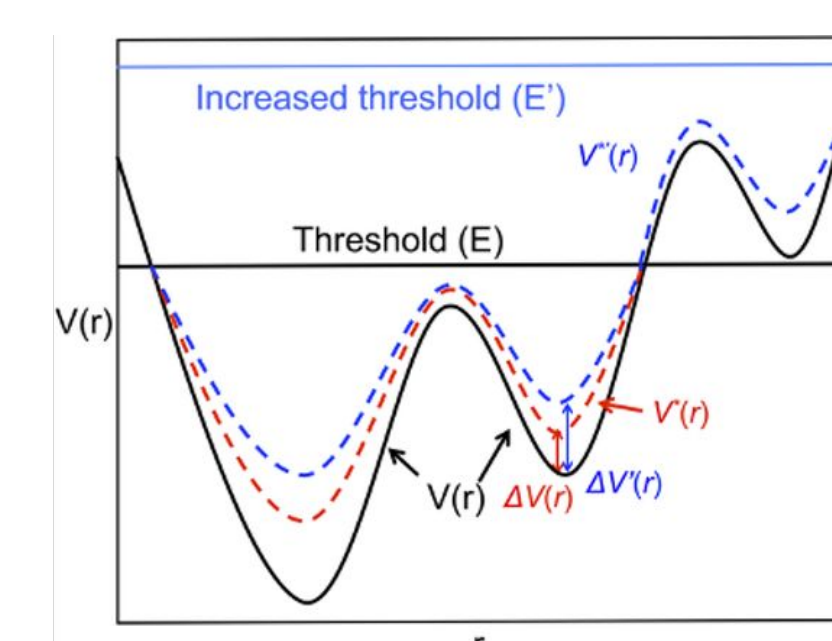
PROCESS DATA
Using Linux, Python, Excel, PyMol

- Start with known multidomain FXR protein
- Deleted selected regions of hinge
- Align truncated molecules with Uniprot sequence and add zincs
- Create 5 predicted structures and select the most likely confirmation

- Simulate solvating complex using lab designed scripts
- Run minimization simulations with varying restraints on energy to stabilize protein
- Simulate heating the complex to physiological temperature
- Run restrained and unrestrained classical Molecular Dynamics for equilibrium simulations

- Run 100 nanoseconds aMD and then create clusters to get more variety in potential simulations
- Run multiple replicates to get more potential variation in simulations
- Combine replicates for analysis

- Strip solvent/ions
- Combine individual replicates into combined trajectory
- Analyze data on trajectories (root mean square fluctuations) and DSSP (categorizes the hinge's secondary structure like the formation of helices throughout the trajectory)



Source:
<https://ambermd.org/tutorials/basic/tutorial4b/>

Figure 8: Accelerated Molecular Dynamics allows proteins to be studied by simulating changing, or restraining, energy barriers of the system.

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