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Modeling and Analyzing Trajectory Differences based on Changes in the Hinge Structure of the FXR Nuclear Receptor

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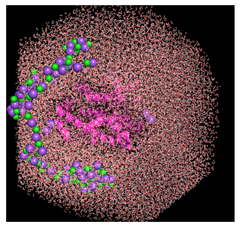


Model of Hinge 1 in solution visualized in PyMOL

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 diseases[4].

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)]

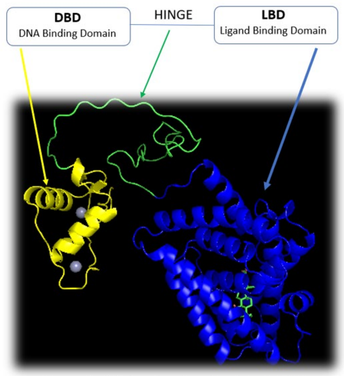


Figure 1: Molecule visualized in PyMOL

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.

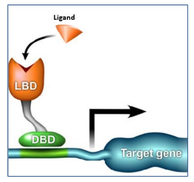


Figure 2: Nuclear receptor

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 hinge[5]. Based on this, the main questions for this study are: Which parts of the hinge, if any, are involved in interdomain interaction between the LBD and the DBD? How does changing the hinge affect the protein's trajectories, salt bridge interactions, and function?

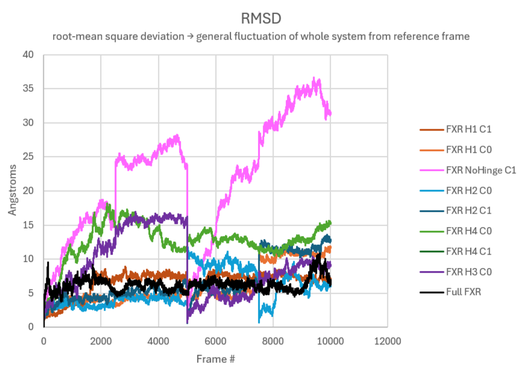
Figure 3: RESIDUE (AMINO ACID) ORDER OF THE FXR DBD-HINGE-LBD

RIKGDELCVVCGDRASGYHYNALTCEGCKGFFRRSITKNAVYKCKN
GGNCVMDMYMRRKCQECRLRKCKEMGMLAEC**MYTGLLTEIQCKS**
KRLRKNVKQHADQTVNEDSEGRDLR**QVTSTTKSCR**EKTELTPDQQ
TLLHFIMDSYNKQRMPQEITNKILKEEFSAEENFLILTEMATNHVQVLV
EFTKKLPGFQTLDHEDQIALLKGSAVEAMFLRSAEIFNKKLPSGHSDL
LEERIRNSGISDEYITPMFSFYKSIGELKMTQEEYALLTAIVILSPDRQY
IKDREAVEKLQEPLLDVLQKLCKIHQPENPQHFACLLGRLTELRTFNH
HHAEMLMSWRVNDHKFTPLLCEIWDVQ

Residues **removed** for each of the hinge "sections"
HINGE 1: **MYTGLLTEIQCKS**
HINGE 2: **KRLRKNV**
HINGE 3: **KQHADQTVNEDSEGRDLR**
HINGE 4: **QVTSTTKSCR**
NO HINGE: Removes the entire colored portion of amino acids

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

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

Figure 6: Compares the FXR protein to versions with no hinge, missing hinge 1 and missing hinge 2.

Figure 7: Compares the FXR protein to versions with no hinge, missing hinge 3 and missing hinge 4.

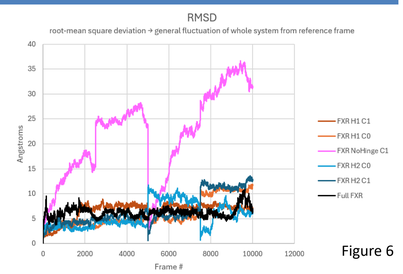


Figure 6

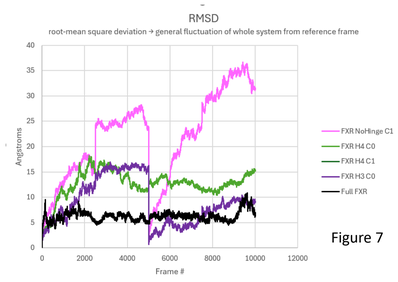
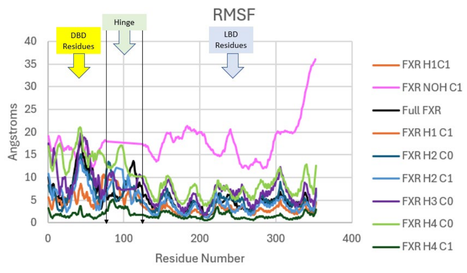


Figure 7



RMSF Graphs: Show the root-mean square fluctuation. This compares the per-residue fluctuation from the reference frame. This gives us an idea of the areas with the most movement.

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

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

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

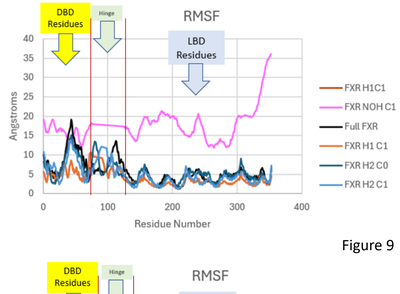


Figure 9

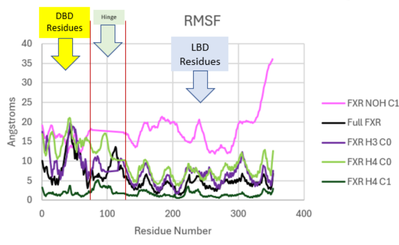
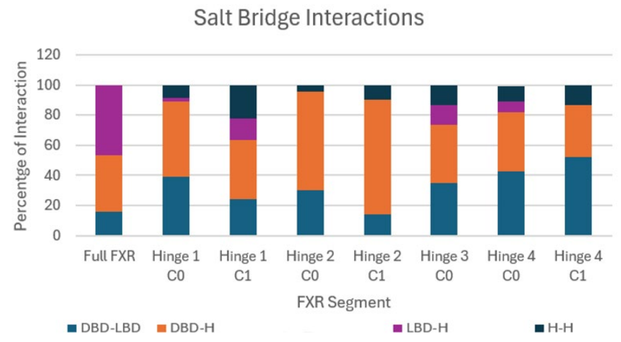


Figure 10

RESULTS



Salt bridges are non-covalent interactions between amino acids in proteins most commonly observed contribution to the stability of unfavorably folded protein conformations. [Figure 11 above]

Most of the salt bridge interactions happened less than 1% of the time, but there were a few simulations showing salt bridges forming more than 10% of the time. These were:

- FXR Hinge 1 C0- GLU242 and ARG62 (LBD-DBD)
- FXR Hinge 2 C0- GLU22 and ARG101 (DBD and hinge)
- FXR Hinge 4 C1- ASP49 and LYS210 (DBD-LBD) AND GLU81 and ARG88 (hinge-hinge)

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.
- There is a difference in salt bridge interactions when portions of the hinge are removed. These interactions could affect the shape of the protein, making it nonfunctional or less functional.

FUTURE RESEARCH

To improve the research, the following should happen:

- Additional aMD should be run to get 500 nanoseconds for depth of data and better analysis
- Complete the modeling of clusters that were unable to be modeled at this time
- Simulate and visualize the same FXR versions with a ligand to see how that affects the trajectories

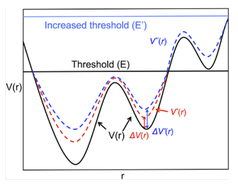
METHODS

MODEL
- Start with a multidomain FXR model (cite Okafor paper)
- Delete selected regions of hinge
- Align truncated molecules with Uniprot sequence
- Use Modeller to create 5 different predictions of the structure and selected most likely conformation

MINIMIZE THE COMPLEX
- Model solvating complex
- Run minimizations with varying restraints on energy to stabilize the protein
- Heat the complex to physiological temperature
- Run restrained and unrestrained classical Molecular Dynamics simulations for equilibration

RUN ACCELERATED MOLECULAR DYNAMICS
- Run 100 nanoseconds aMD and then clustered
- Use the top two clusters to run 500 ns aMD simulations
- Collect outputs of the trajectories of the molecule
- Run multiple reps, which will later be combined for analysis

PROCESS TRAJECTORIES
- Strip solvents/ions
- Combine individual results into a combined trajectory
- Analyze data



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

Figure 4: Accelerated Molecular Dynamics allows proteins to be studied by simulating changing, or restraining, energy barriers of the system.

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