Computational Investigation of Isoform Selectivity in Liver X Receptors

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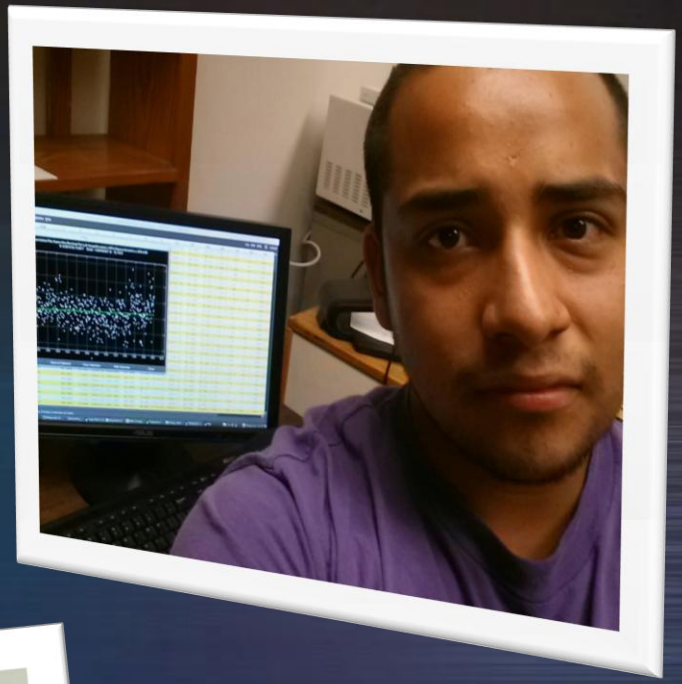
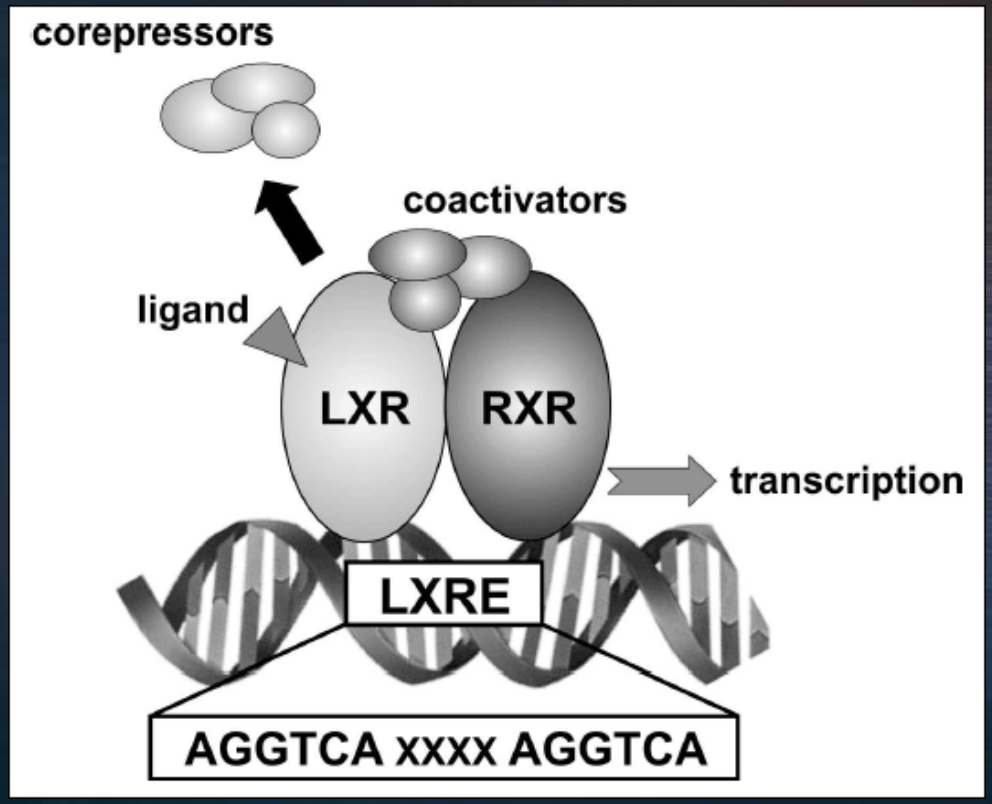
Presentation Outline

- Introduction
- Theory
- Methods
- Results
- Conclusion



Introduction

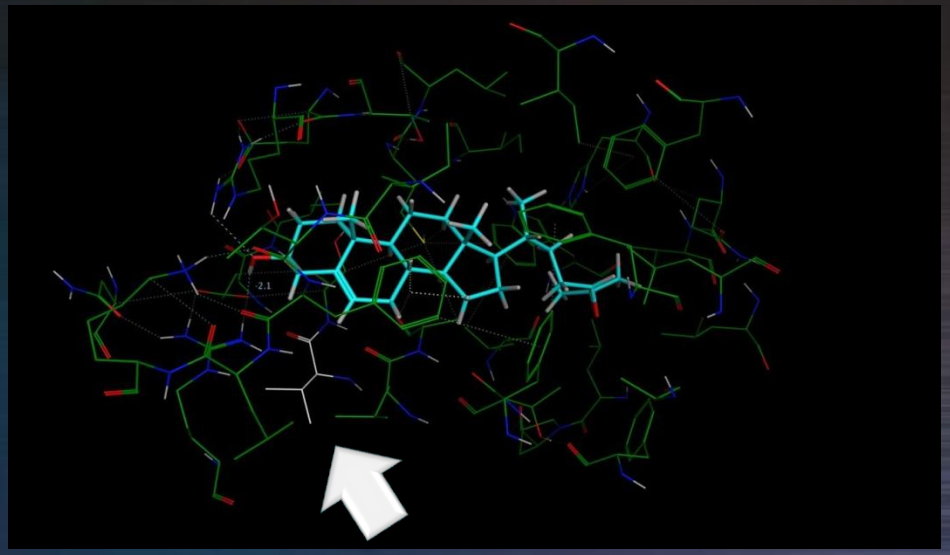
- Liver X Receptor
  - Nuclear Receptor
  - Forms heterodimer with Retinoid X Receptor (RXR)
  - α and β isoforms
    - α is found in Liver
    - β is expressed everywhere
  - Binds to Cholesterol Derivatives
  - Induces Reverse Cholesterol Transport (RCT)

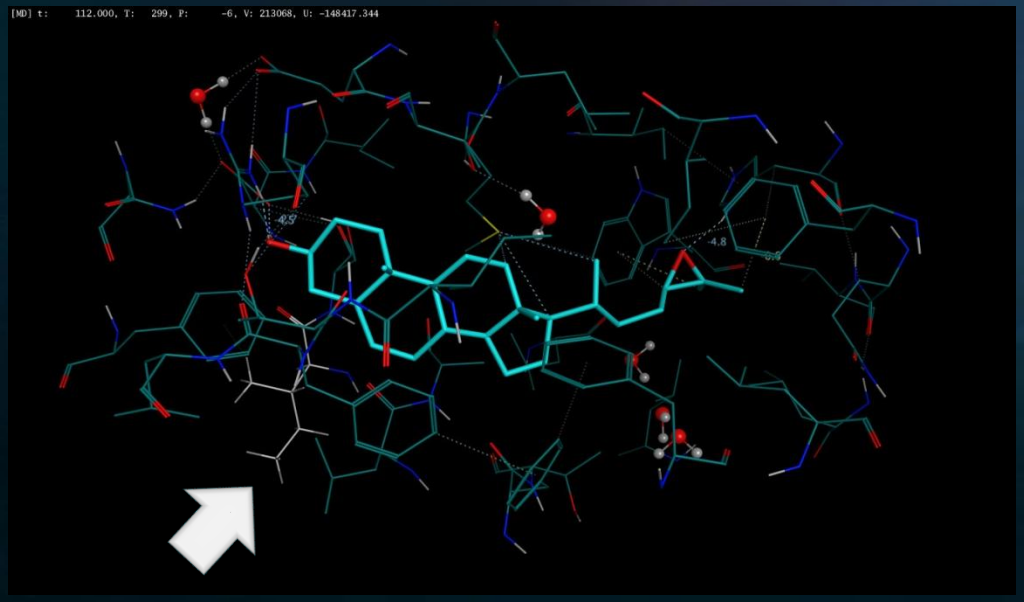


http://www.jpp.krakow.pl/journal/archive/12_08_s7/articles/03_article.html

Introduction

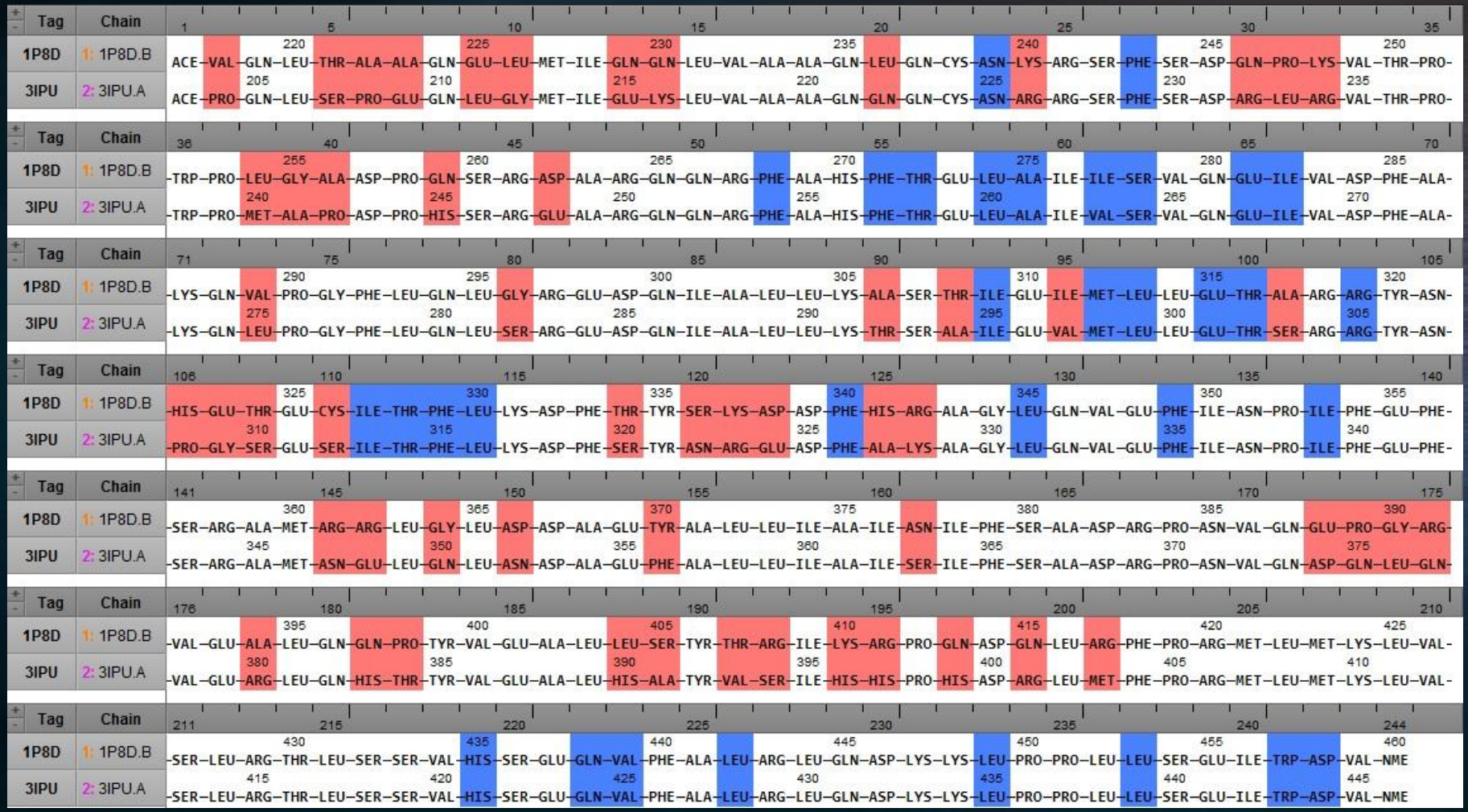
- Binding Pocket
  - Large Hydrophobic Pocket
  - Highly Conserved Ligand Binding Doman (LBD)
  - Flexible
  - One Amino Acid Difference





Binding Pocket Difference:
LXRα: Valine
LXRβ: Isoleucine

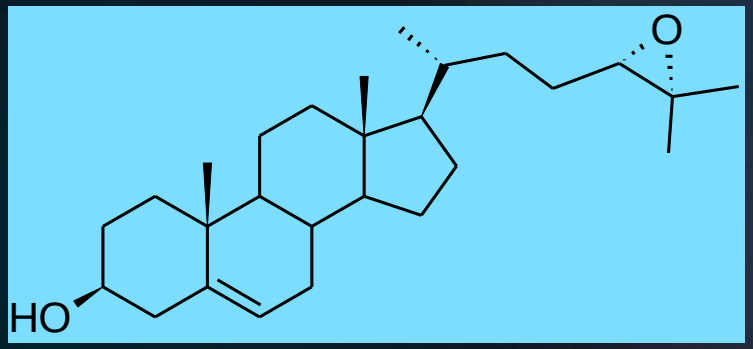
Introduction



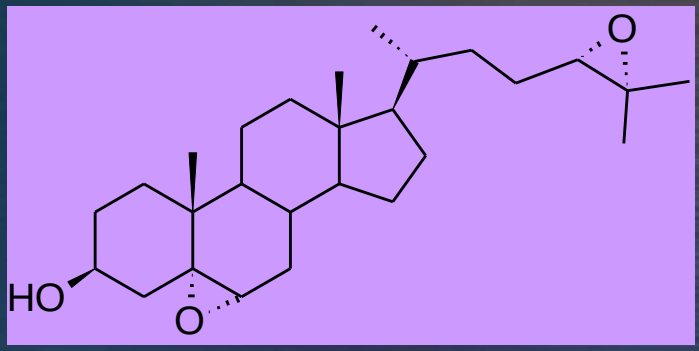
Introduction

- Goal: Investigate Ligand Selectivity

24(s),25-EpoxyCholesterol (EC)



VS



5,6-24(s),25-DiEpoxyCholesterol (DEC)

- DEC is LXRα selective.

Introduction

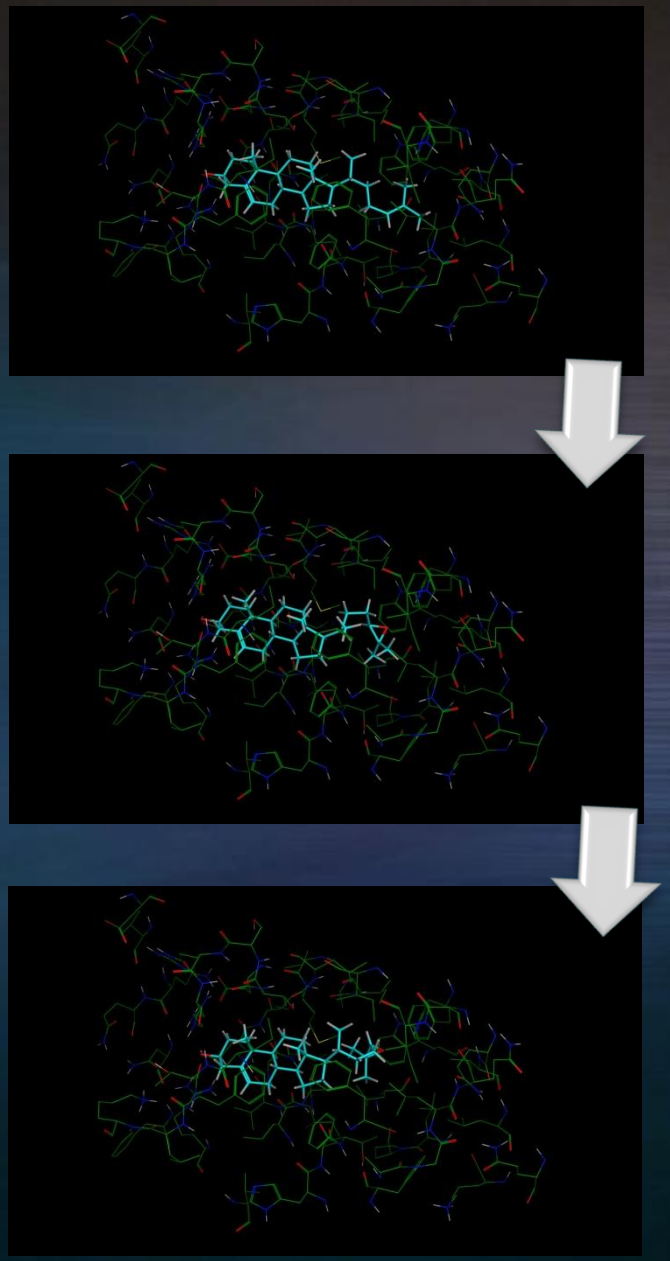
- Question: What determines selectivity?
  - Protein Geometry or Ligand Interaction
- Computational investigations using Molecular Operating Environment (MOE)
  - Docking
  - Molecular Dynamics
  - Glycine Scanning

Introduction:

- Ligand-Protein Interactions
- Study Geometry of Complex
- Find Binding Mechanism
- Design selective drugs
- Fight Cancer!

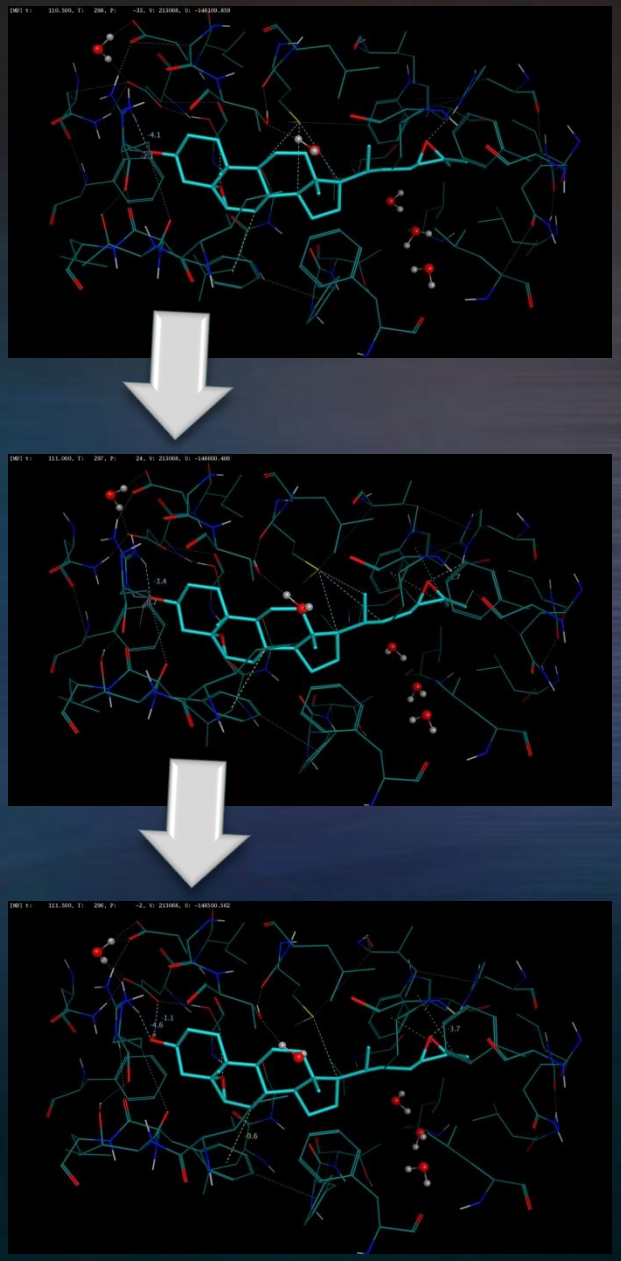
Introduction

- Docking – holds receptor static, not helpful
- Induced Fit Docking – allows receptor movement
  - Scores lowest binding energy (kcal/mol)
  - Generates Poses, scored by lowest energy state

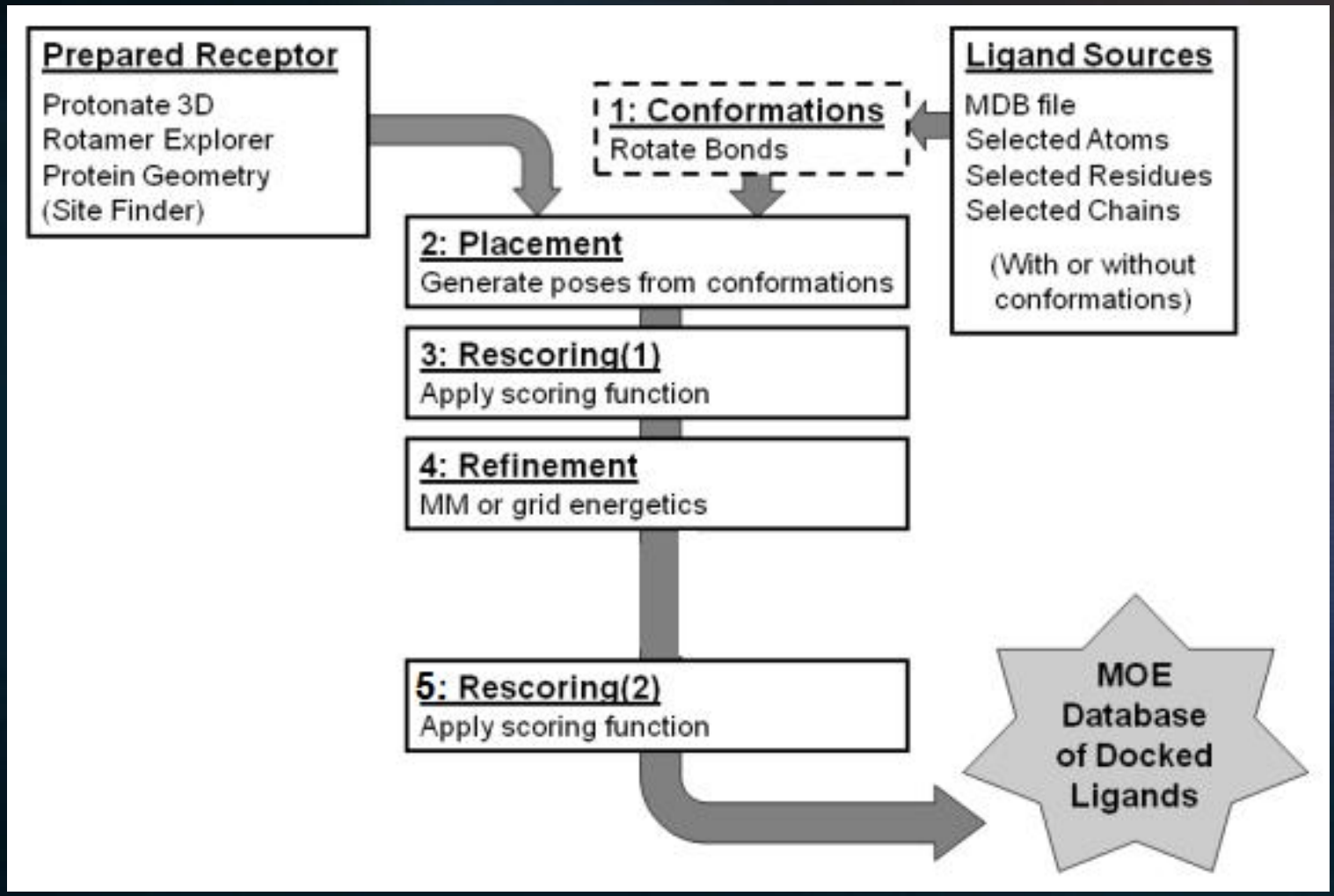


Introduction

- Molecular Dynamics
  - Simulate movement
  - Multiple Poses
  - Computationally expensive
  - Sensitive to Systems with High Degrees of Freedom
- Glycine Scanning
  - What individual amino acids contribute to binding?



Theory: Induced Fit Docking



Theory

- Induced Fit Docking
  - 1. Placement
    - Alpha Triangle – random
    - Triangle Matcher – slightly systematic

Theory

- 2. Scoring
  - Lower Scores Indicate more favorable poses
  - London dG Scoring
    - Estimates ΔG_{bind} of Ligand/Receptor from Pose

$$\Delta G = c + E_{flex} + \sum_{h-bonds} c_{HB} f_{HB} + \sum_{m-lig} c_M f_M + \sum_{atoms\ i} \Delta D_i$$

    - c is average gain/loss of rotational & translation entropy
    - ΔE_{flex} is energy lost due to flexibility of ligand
    - ΔD is the desolvation energy of an atom

Theory

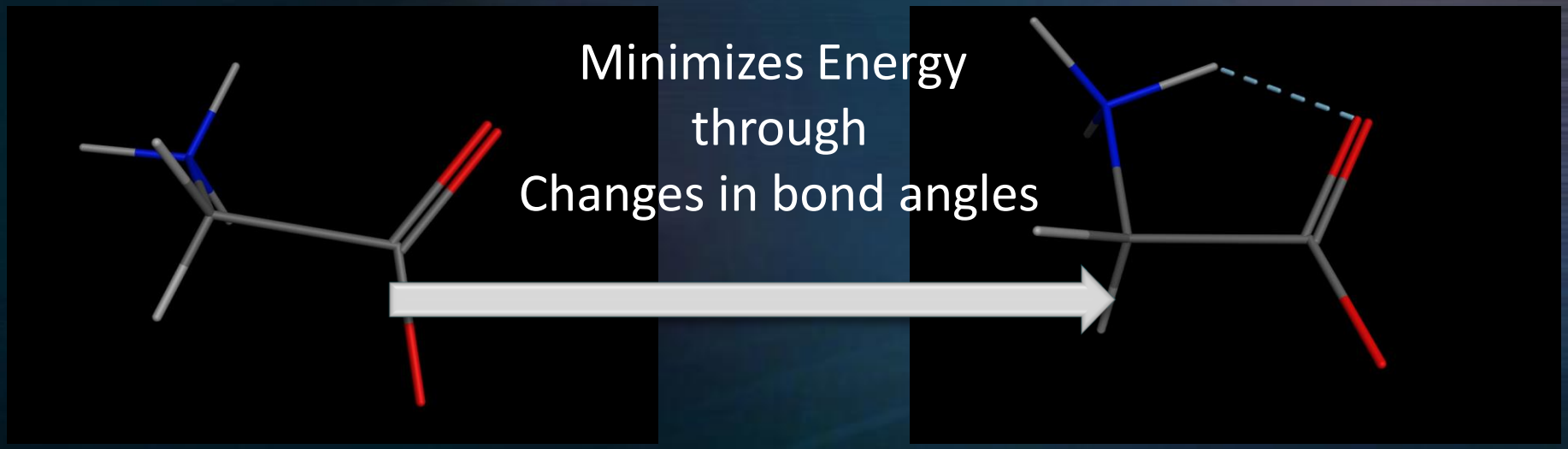
- 2. Scoring
  - GBVI/WSA Scoring
    - Estimates from given poses

$$\Delta G \approx c + \alpha \left[\frac{2}{3}(\Delta E_{coul} + \Delta E_{sol}) + \Delta E_{vdw} + \beta \Delta SA_{weighted} \right]$$

    - c is average gain/loss of rotational & translation entropy
    - α and β are constants; force field dependent
    - ΔE_{coul} is the coulombic electrostatic term,
    - ΔE_{sol} is the energy contributed by solvent
    - ΔE_{vdw} is the van der Waals contribution to binding
    - SA is surface area weighted by exposure

Theory

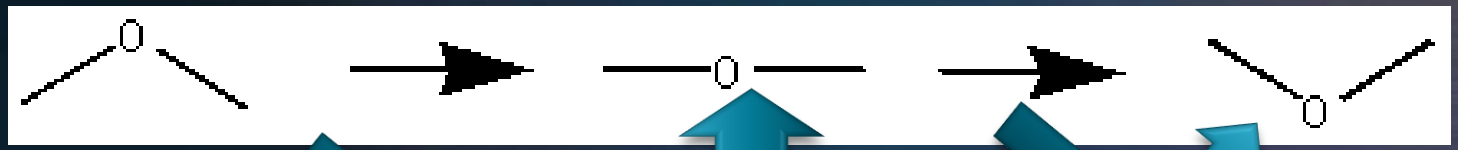
- 3. Refinement
  - Relaxes Strain in System



- 4. Output
  - S Value: Final Score to indicate binding free energy

Theory: Molecular Dynamics

- Molecular Dynamics (MD)
  - Energy Minimization Simulates Molecular Movement

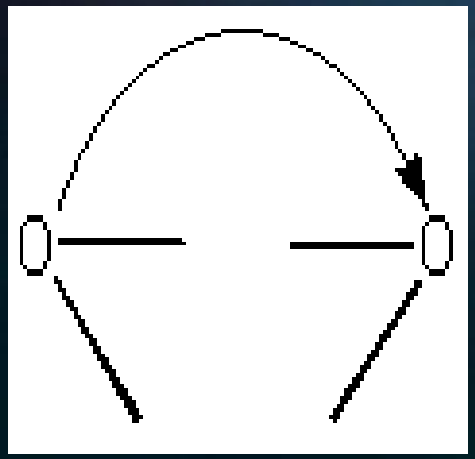


Multiple Changes In Bond Angle

Highly Strained

Relieves Strain

Third Dimension Holds Energy Still Rotates

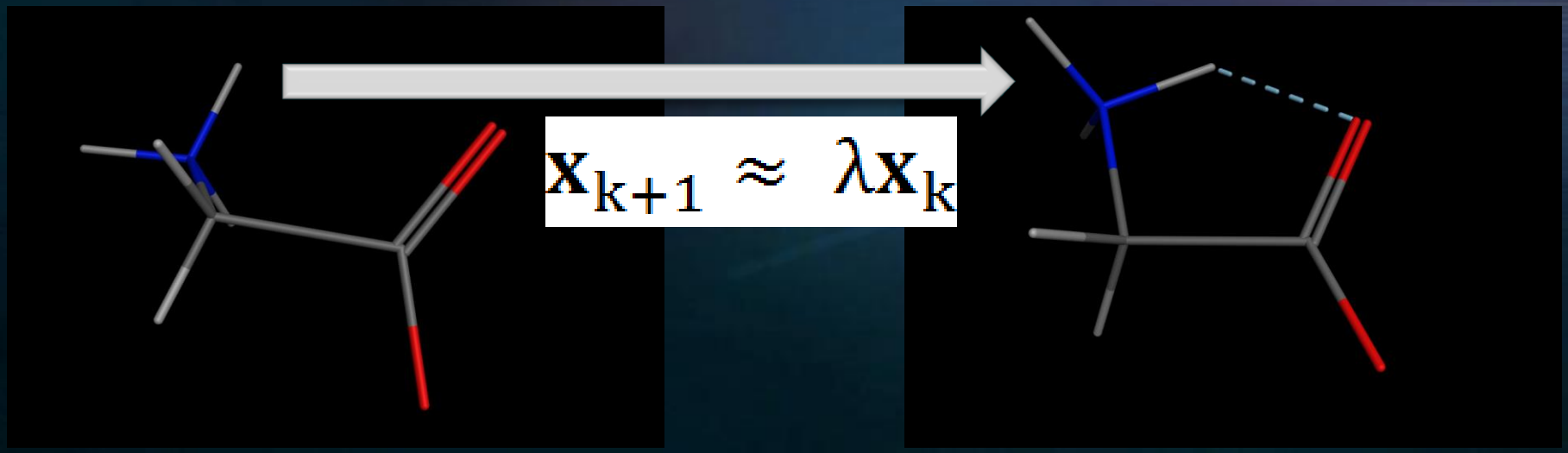


Theory

- System Minimized
  - Fourth Dimension allows further ΔU minimization

$$E(x) = E_{str} + E_{ang} + E_{stb} + E_{oop} + E_{tor} + E_{vdw} + E_{ele} + E_{sol} + E_{res}$$

  - Step-Wise Optimization



Theory

- MD Parameters
  - Solvation
    - Introduce a solvent sphere within 4 Å radius of protein
  - Equilibrate
    - Hold time still, minimize system at a given temperature
  - Production
    - Isothermal & Isochoric
    - Simulates Molecular Movement for *t* picoseconds (ps)

Theory

- Molecular Dynamics

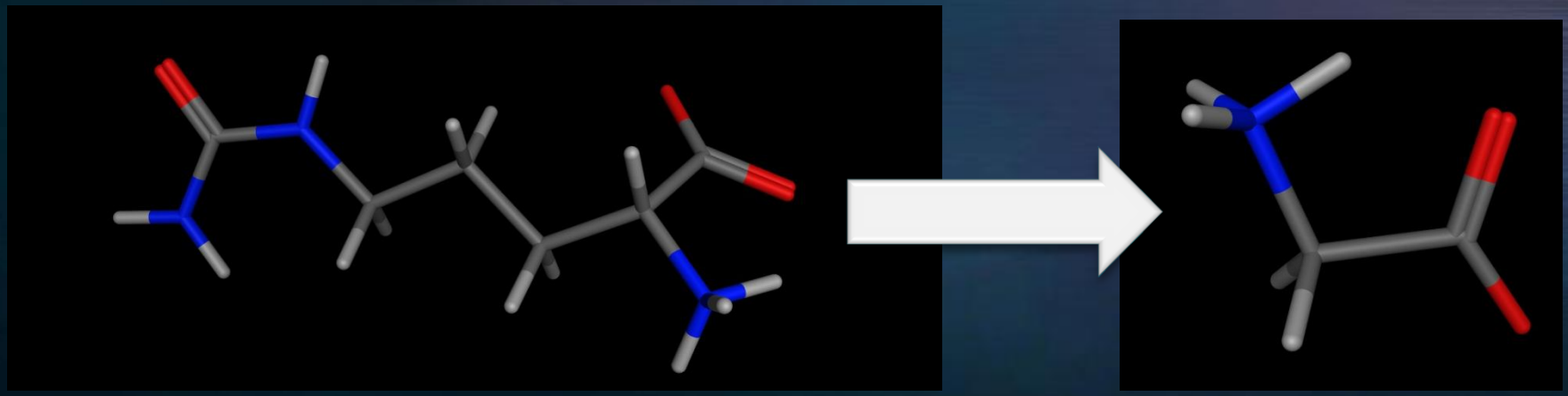
$$H(t) = s\left[\tilde{H}(t) - \tilde{H}(0)\right]$$

$$\tilde{H}(t) = \frac{\mathbf{u}\cdot\mathbf{M}^{-1}\mathbf{u}}{2s^2V^{2/3}} + \frac{u^2}{2Q_T} + \frac{v^2}{2Q_P} + U(V^{1/3}\mathbf{q}) + gkT\log s + PV$$

  - Nosé-Poincaré-Andersen (NPA) equations of motion
  - Utilizes, scaled-space coordinates, real space coordinates, real spaced momenta to describe movement.

Theory: Glycine Scanning

- Glycine Scanning
  - Determination of Relative Binding Contribution
  - Changes Native Amino Acids → Glycine (R=H)

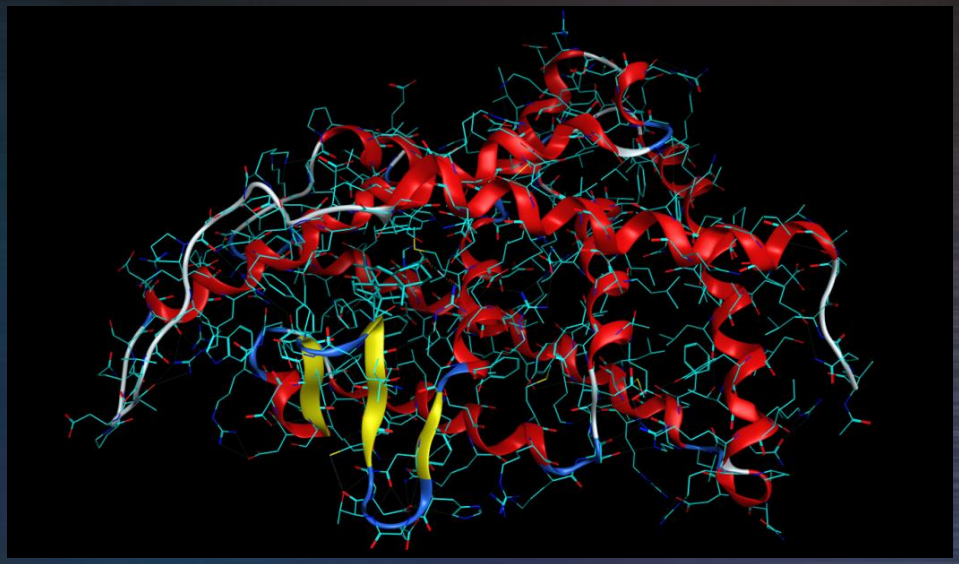


  - Re-Score the Mutated Protein

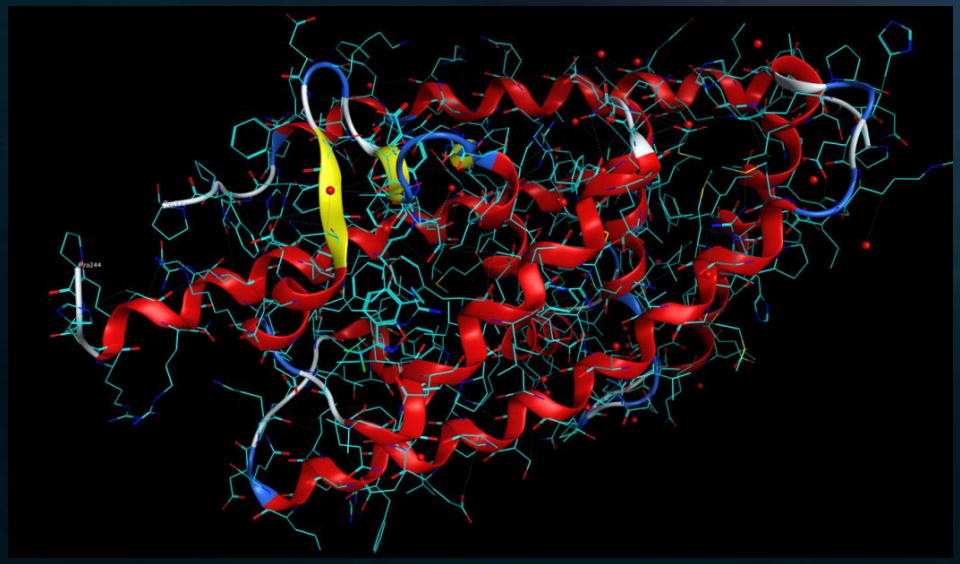
Methods

- Obtained Crystal Structures from Protein Data Bank

IP8D - LXRβ

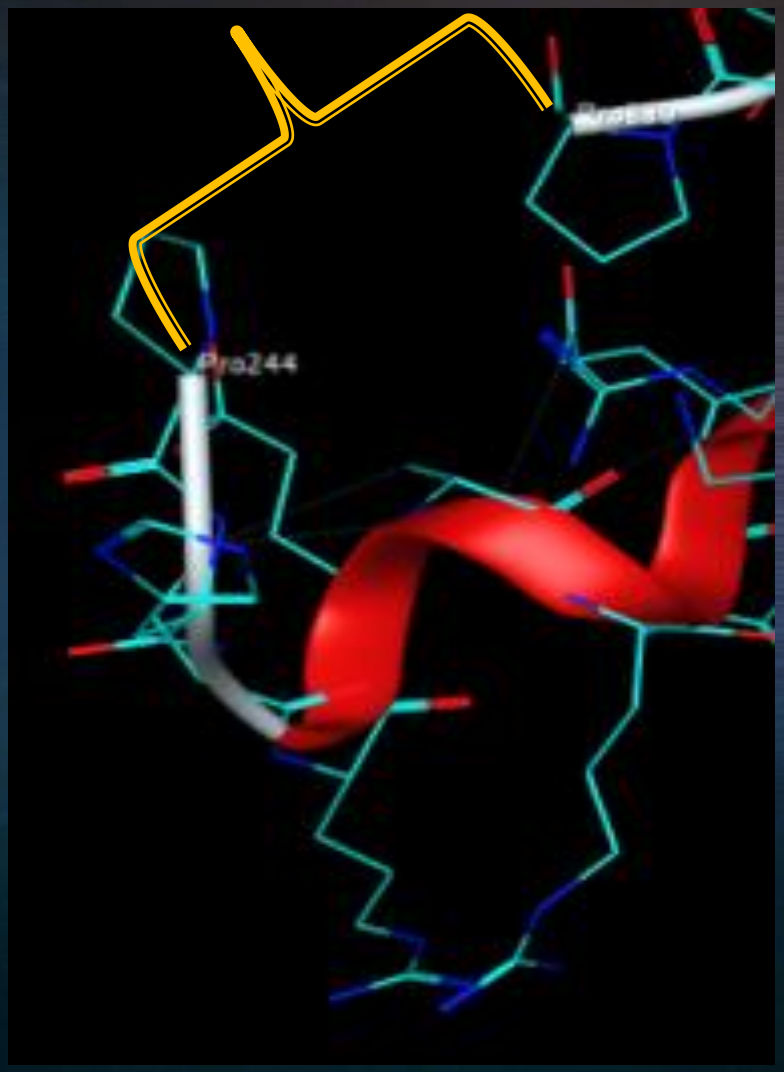


3IPU – LXRα



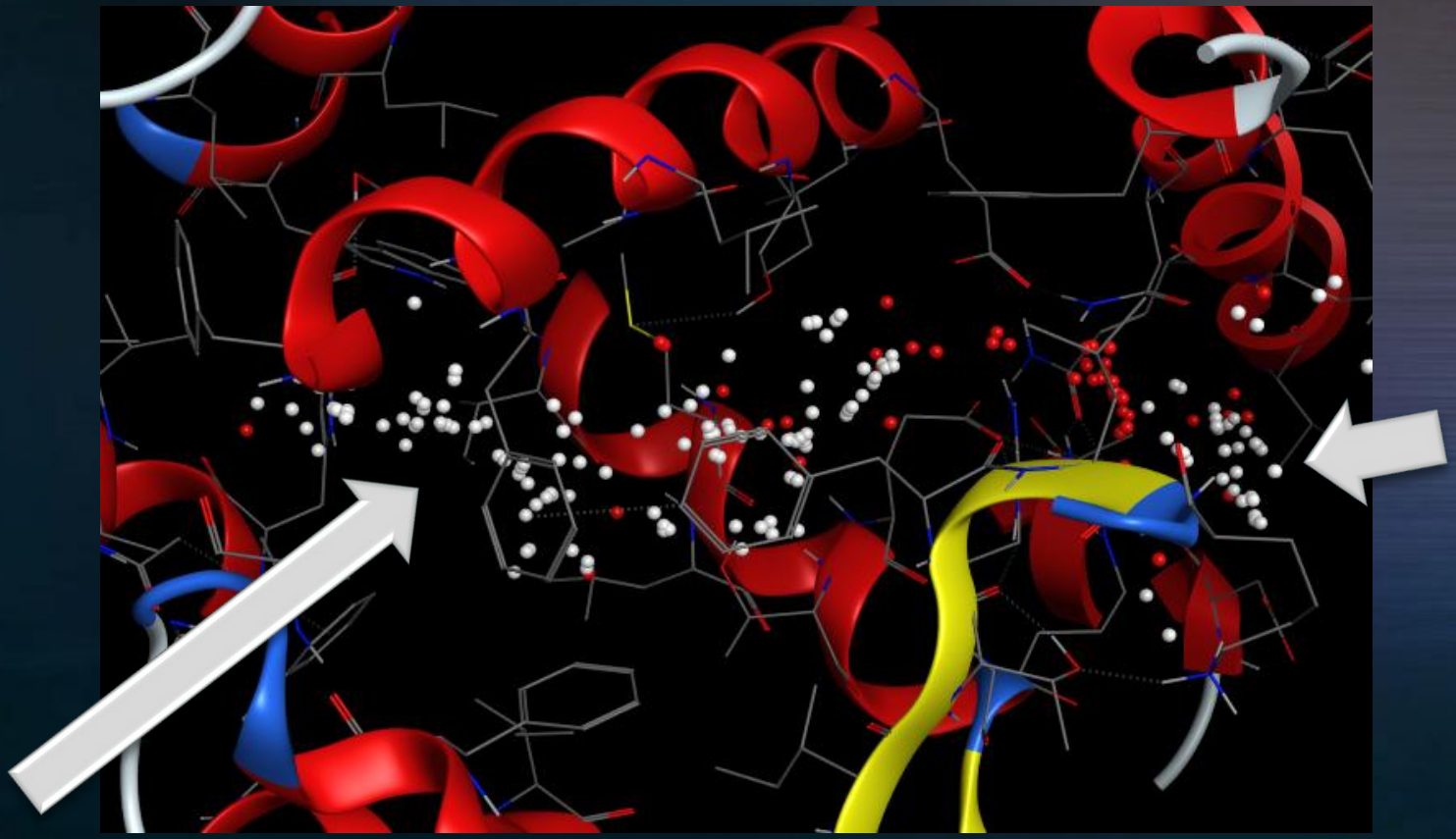
Methods:
Structure Preparation

- Filled in gaps in crystal structure
- Fixed charges
- Protonated
- Minimized



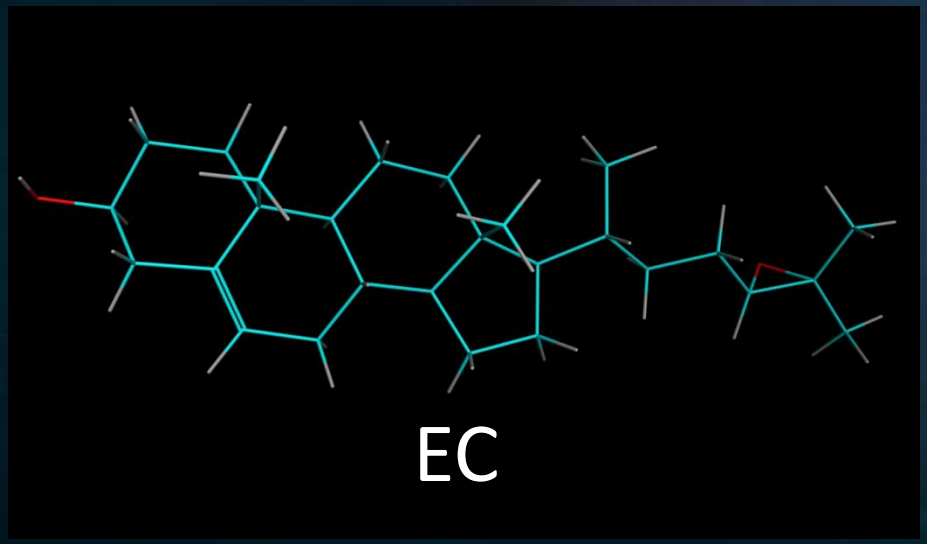
Methods

- Defined the active site

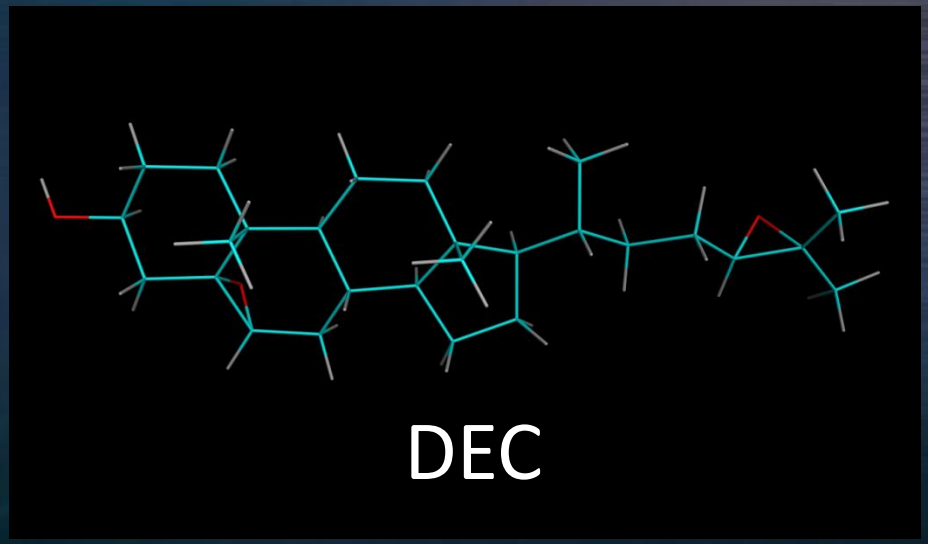


Methods: Ligand Design

- Built:



EC

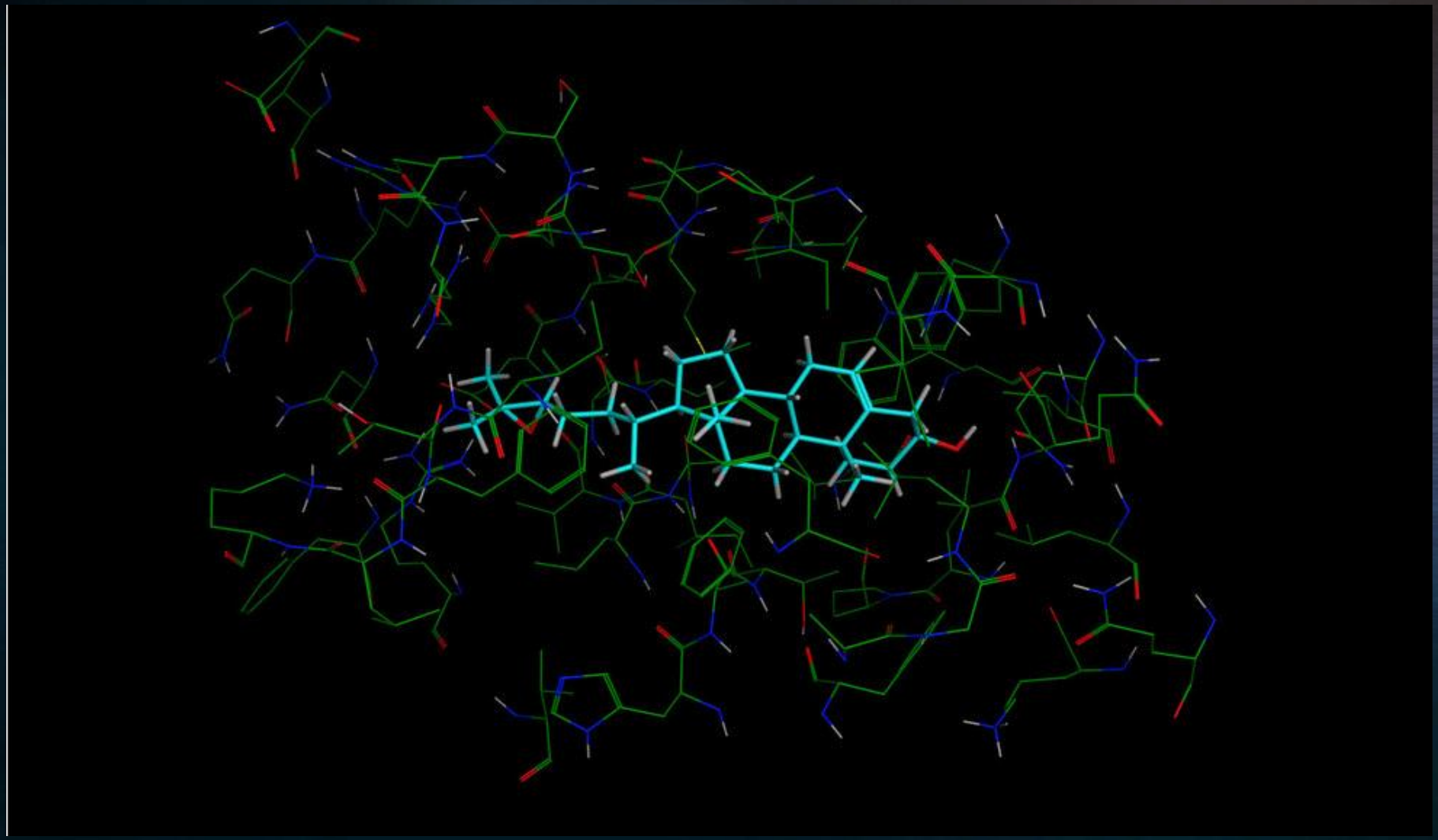


DEC

Methods: Docking

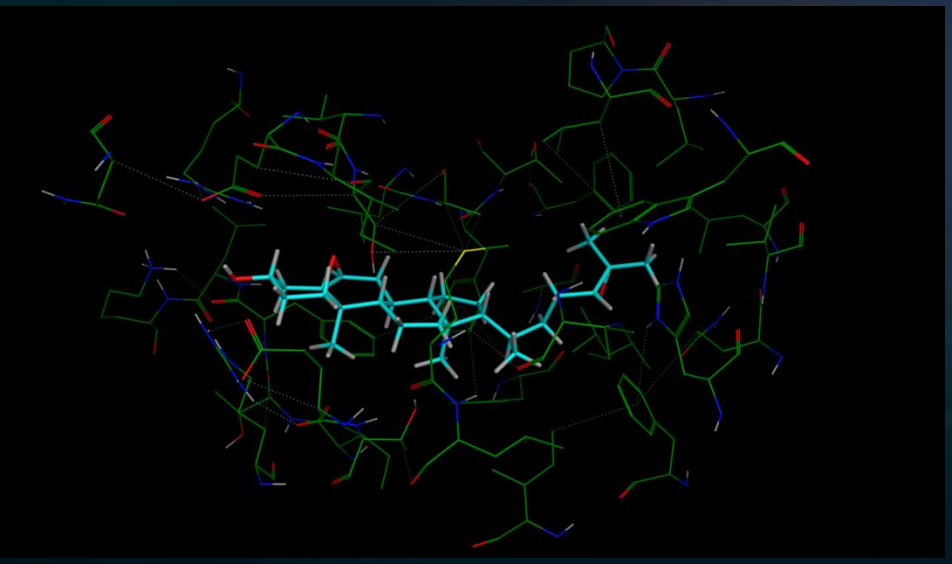
- Used Induced Fit docking to dock EC and DEC to LXRα and LXRβ
- Placement: Triangle Matcher
- Rescoring 1: London dG
- Refinement: Force field: AMBER99
- Rescoring 2: GBVI/WSA dG

Methods: Docking

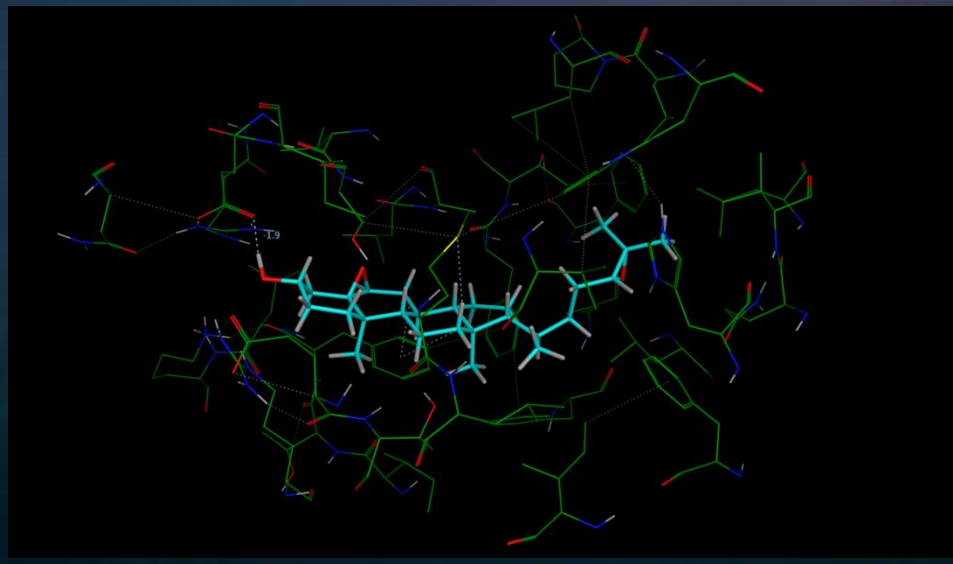


Methods

- Minimized the poses with the 5 lowest S-values
- Re-scored poses; Static Receptor + Ligand
  - GVI/WSA Scoring Algorithm



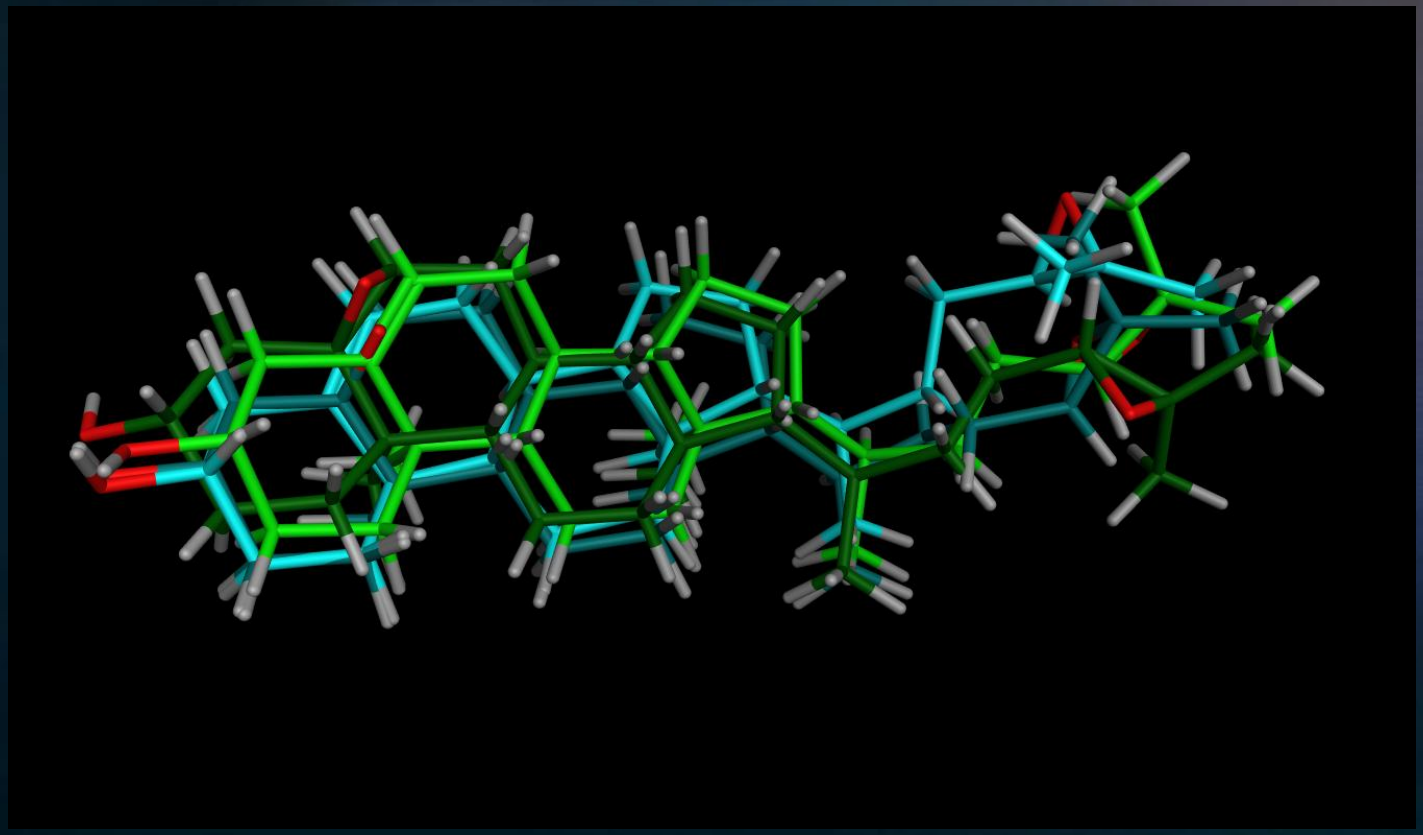
Non-minimized



Minimized

Methods

- Top poses were aligned to compare



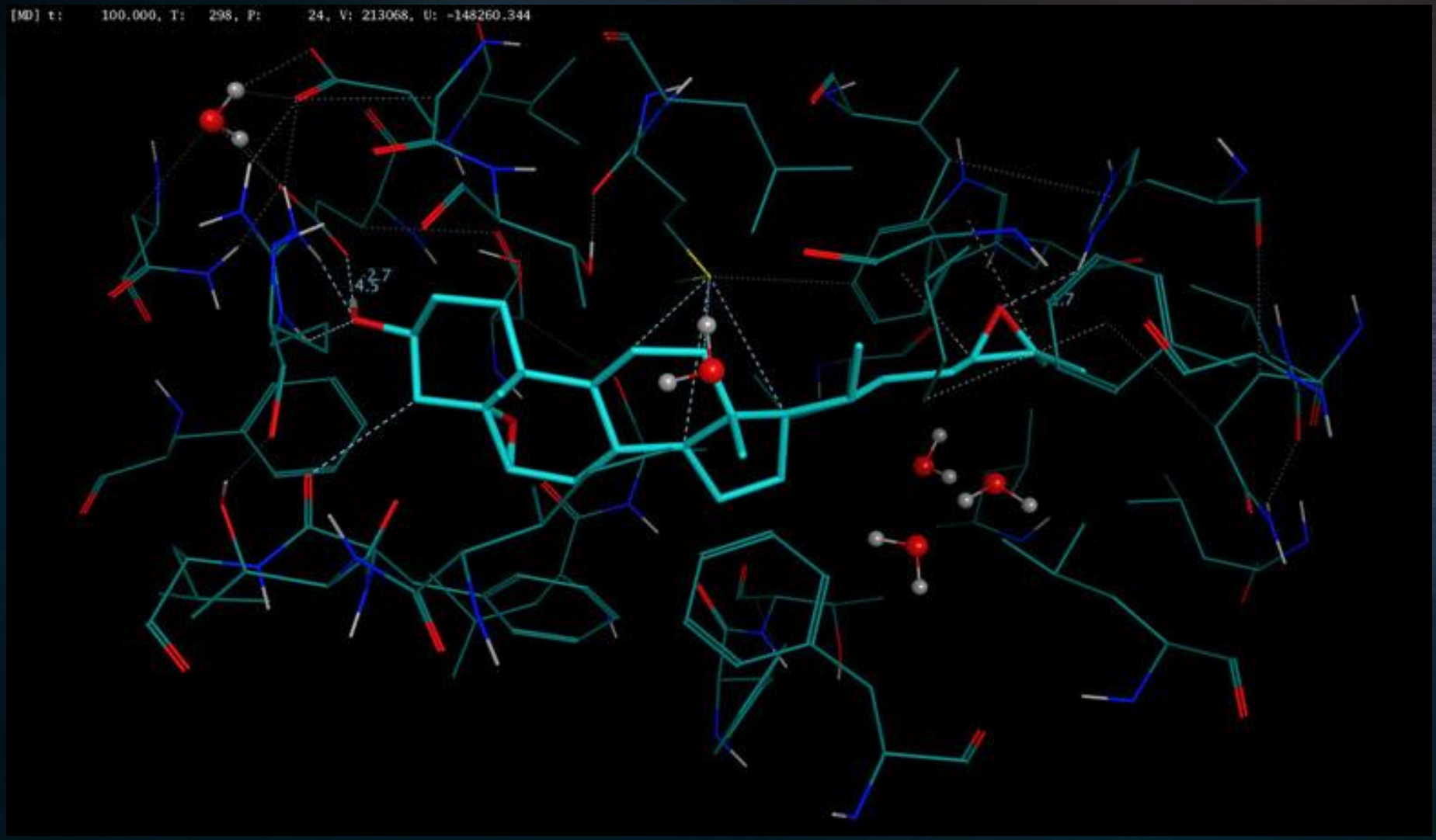
Green-1P8D

Blue-3IPU

Methods: Molecular Dynamics (MD)

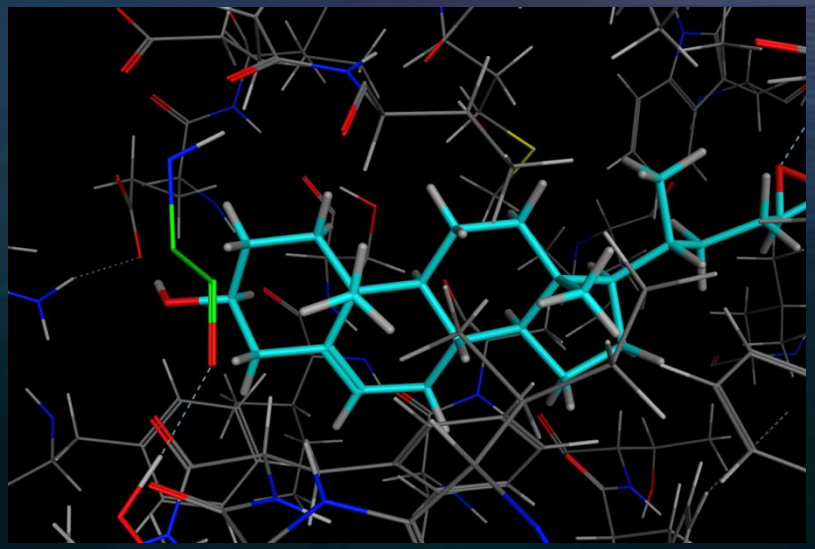
- Ran dynamics for top docking poses in solvent
- Time step 0.002 ps
- Equilibrium stage 100 ps
- Used Nosé-Poincaré-Andersen (NPA) equations of motion
  - Simulated for 500 ps
- Total time 600 ps

Methods: MD



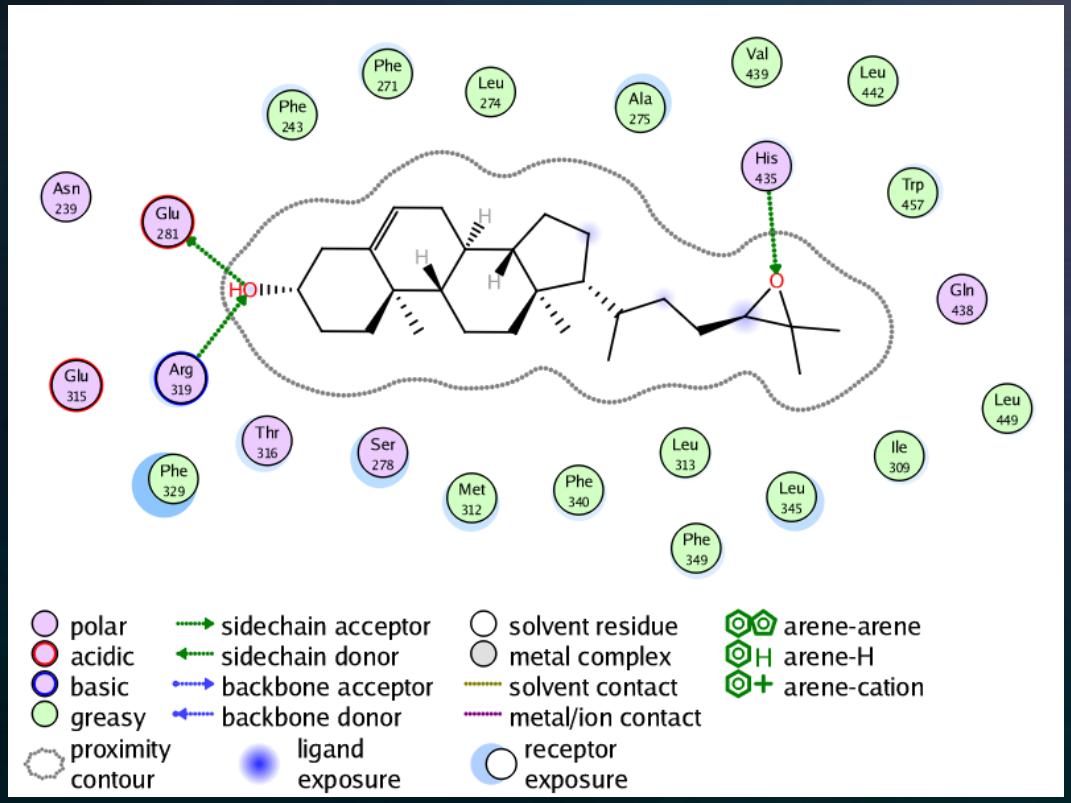
Glycine Scanning

- Glycine scanning for best docked poses and snapshots from dynamics:
  - LXRα – EC
  - LXRα – DEC
  - LXRβ –EC
  - LXRβ –DEC

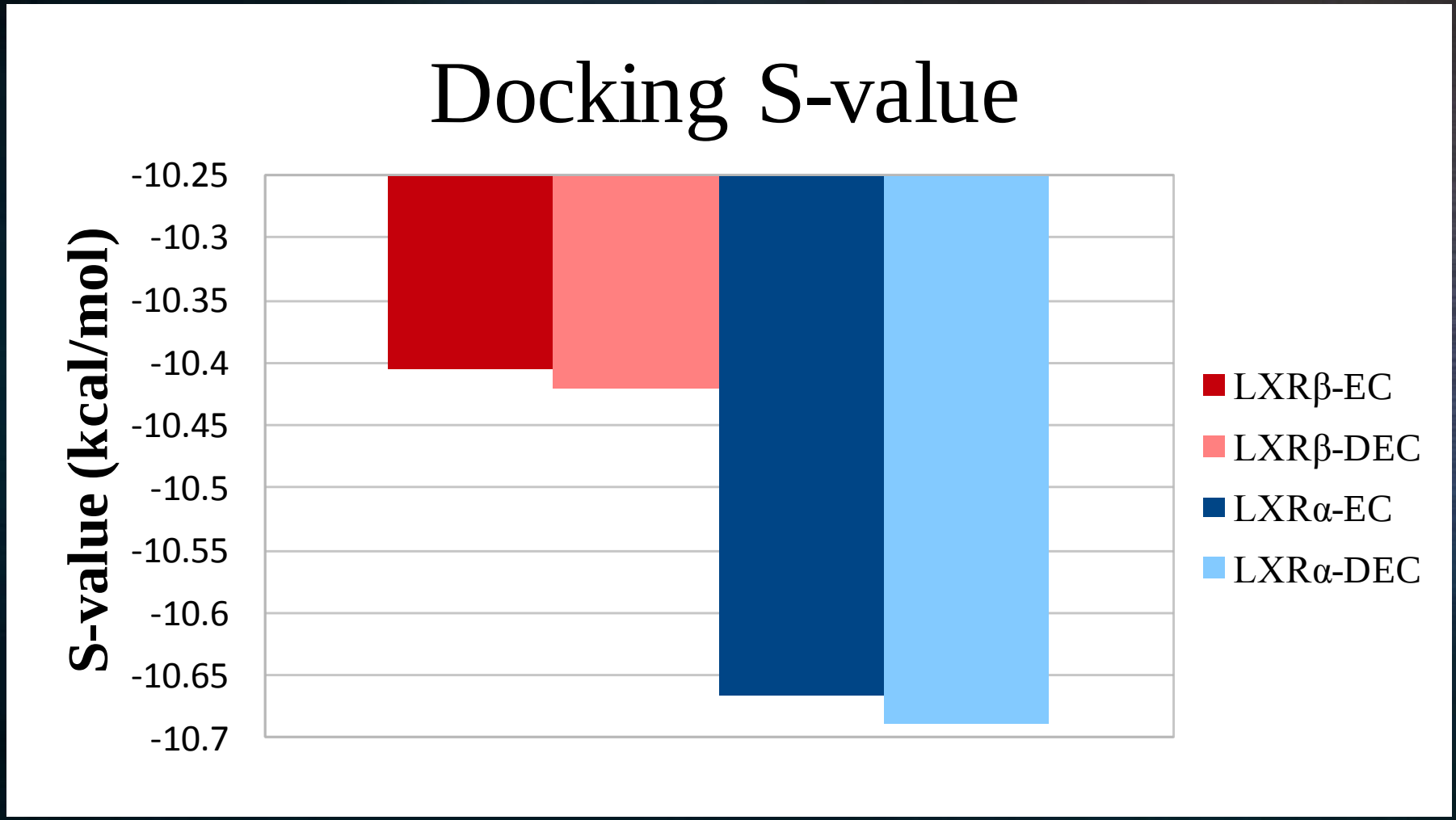


Glycine Scanning

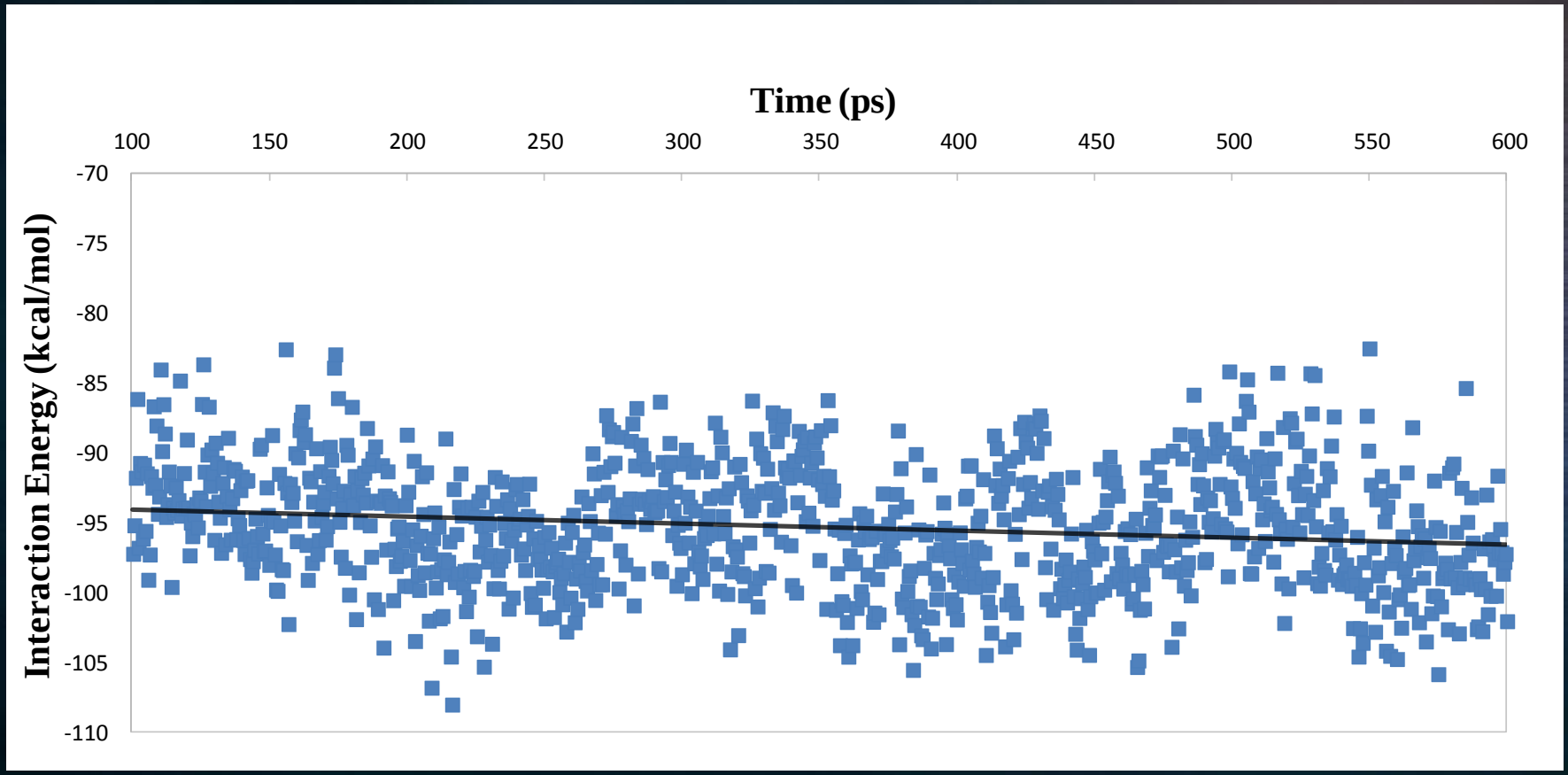
- Used 2-D interaction map to find amino acid/ligand interactions
- Individually mutated amino acid to Glycine and then Re-scored



Results - Docking

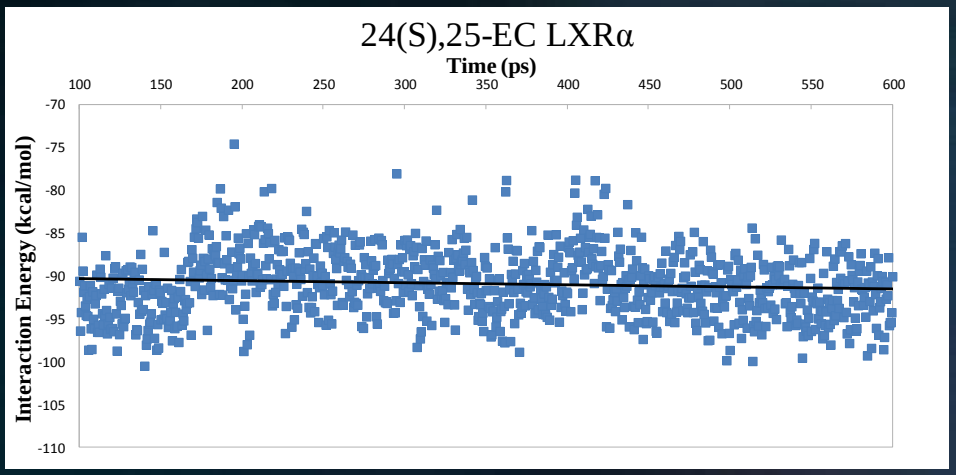


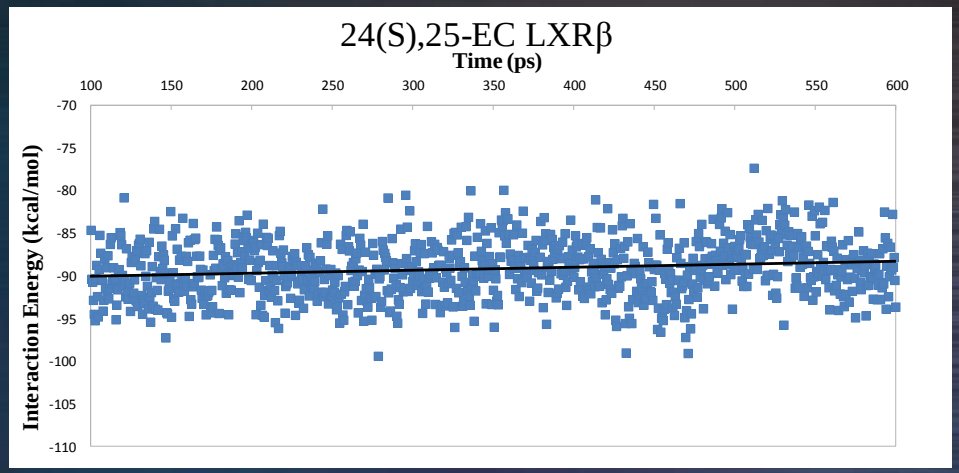
Interaction Energy vs. Time Graph

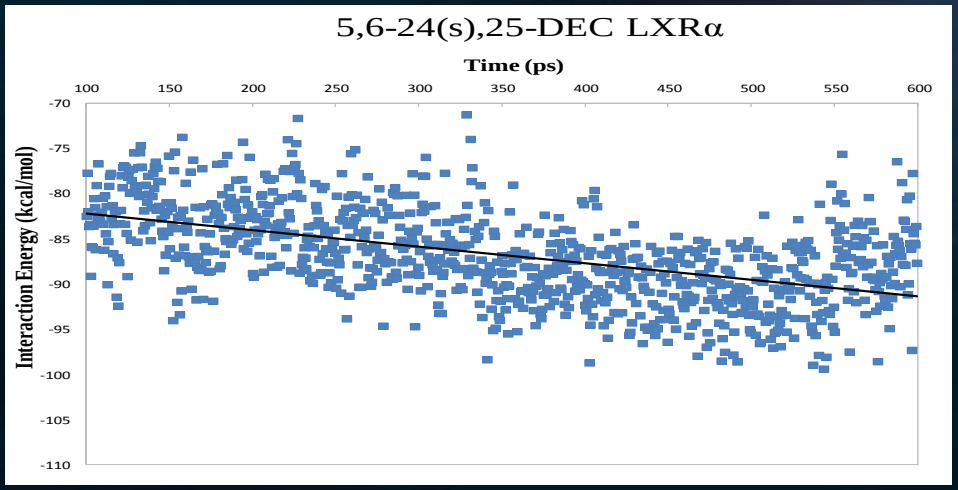


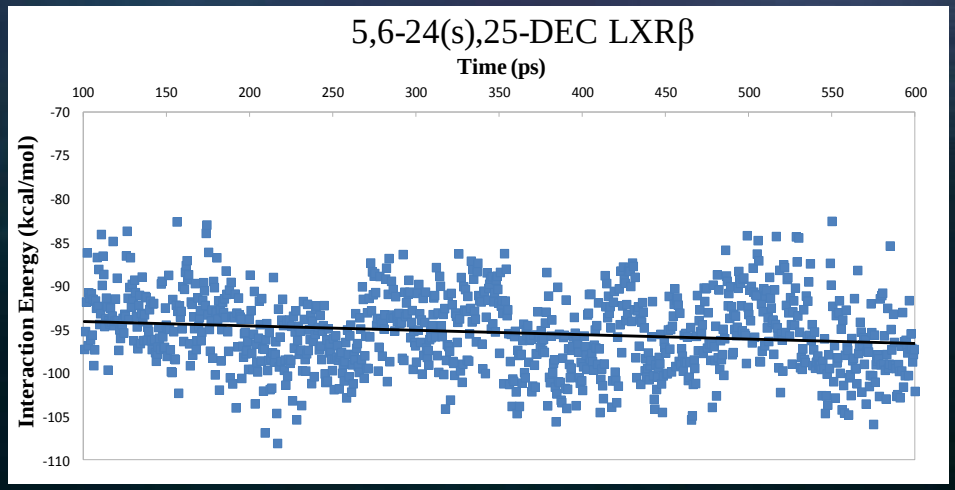
Results - Dynamics

- Potential Interaction Energy



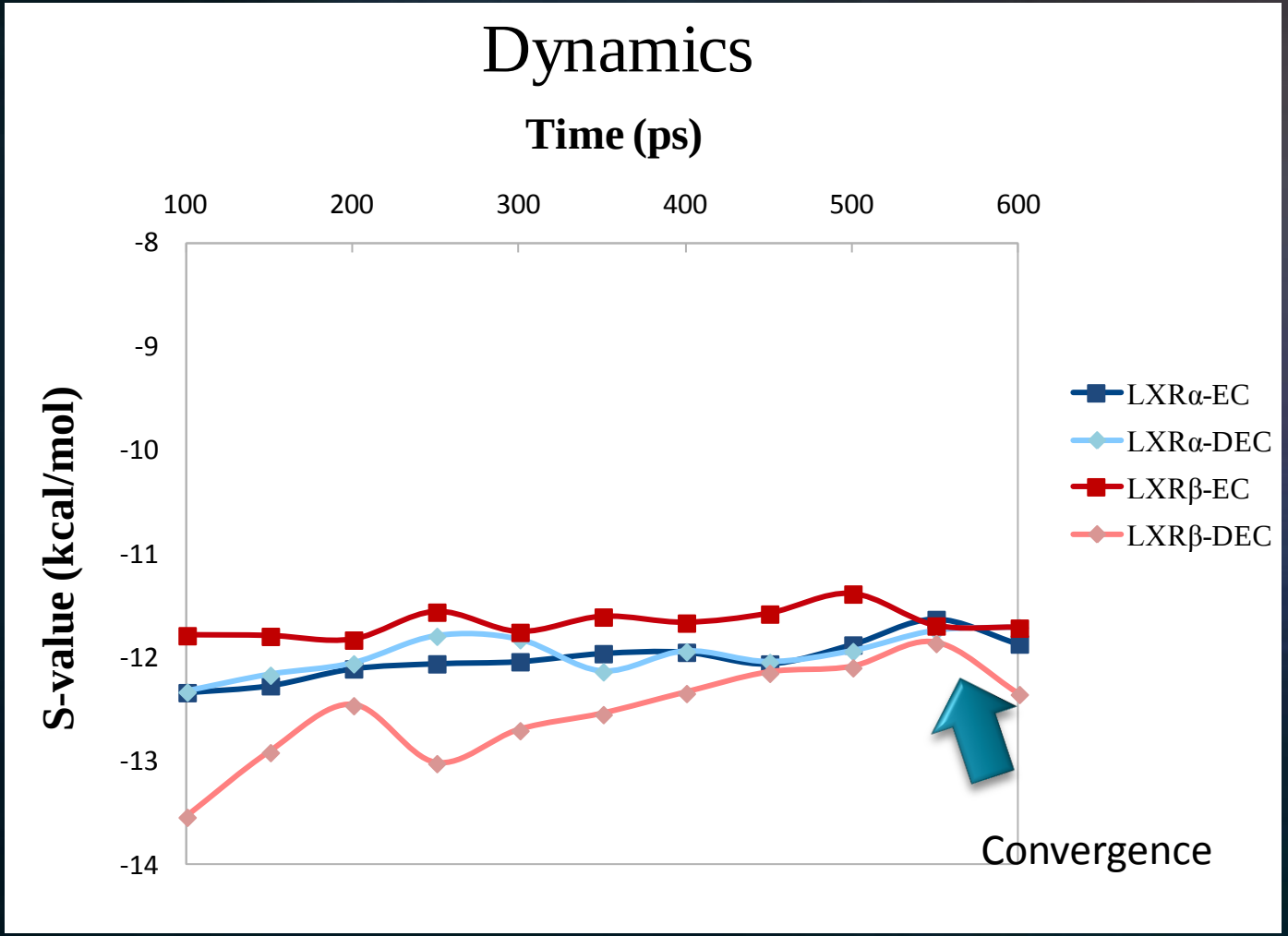






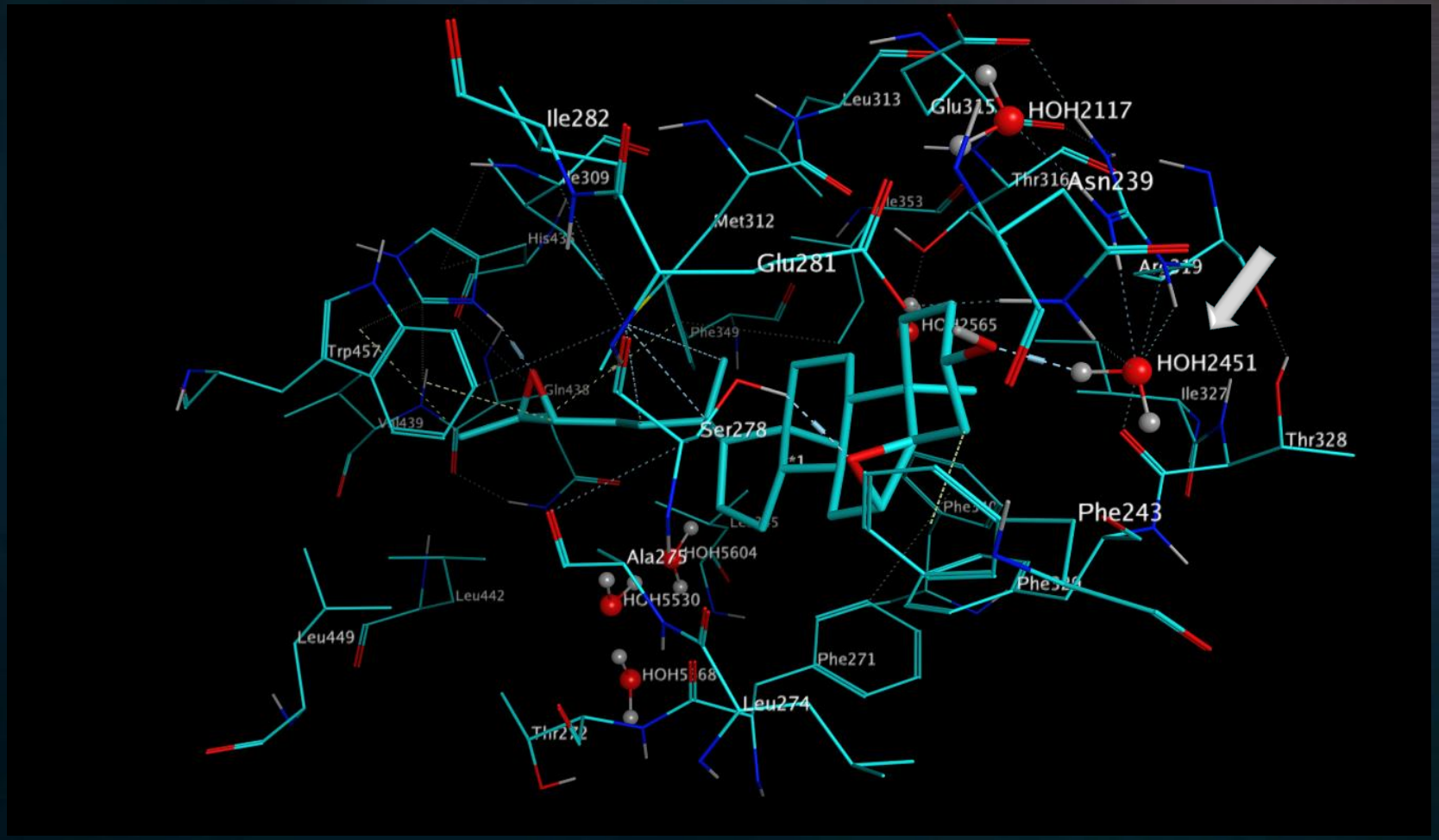
Results-Dynamics

- Snapshot Re-score



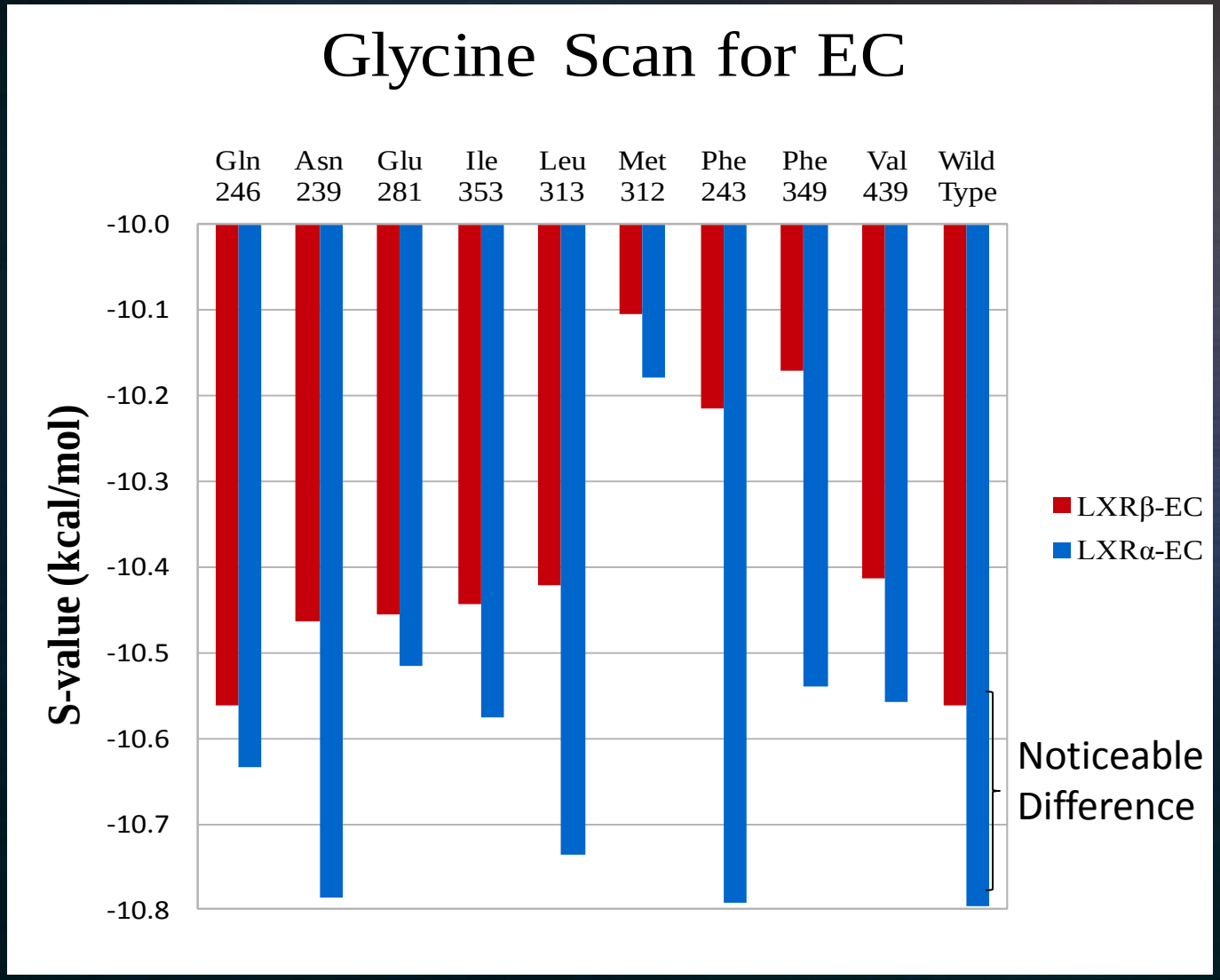
Results - Dynamics

- Web of hydrogen bonds

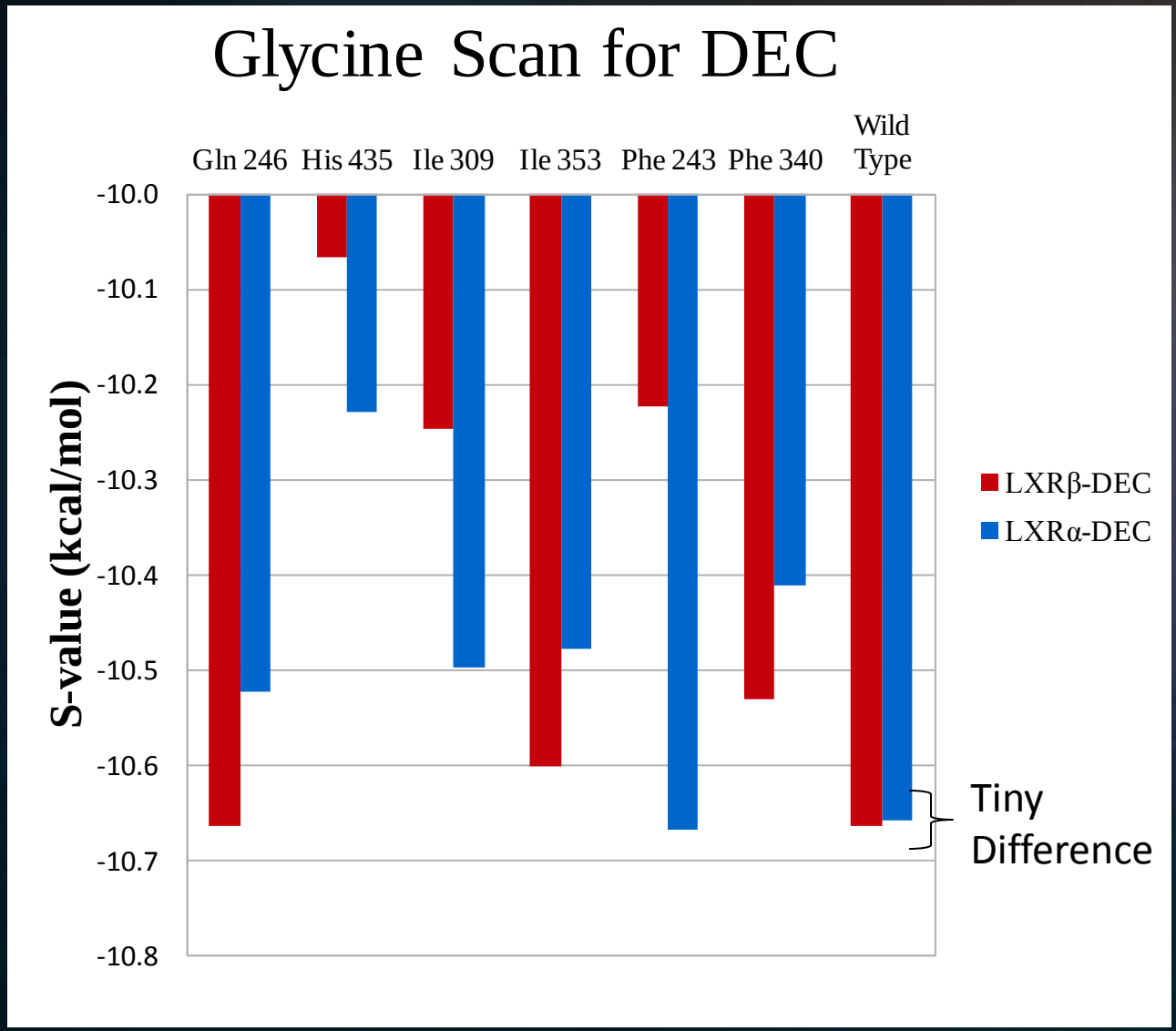


Results-Glycine Scanning

- Significant amino acids

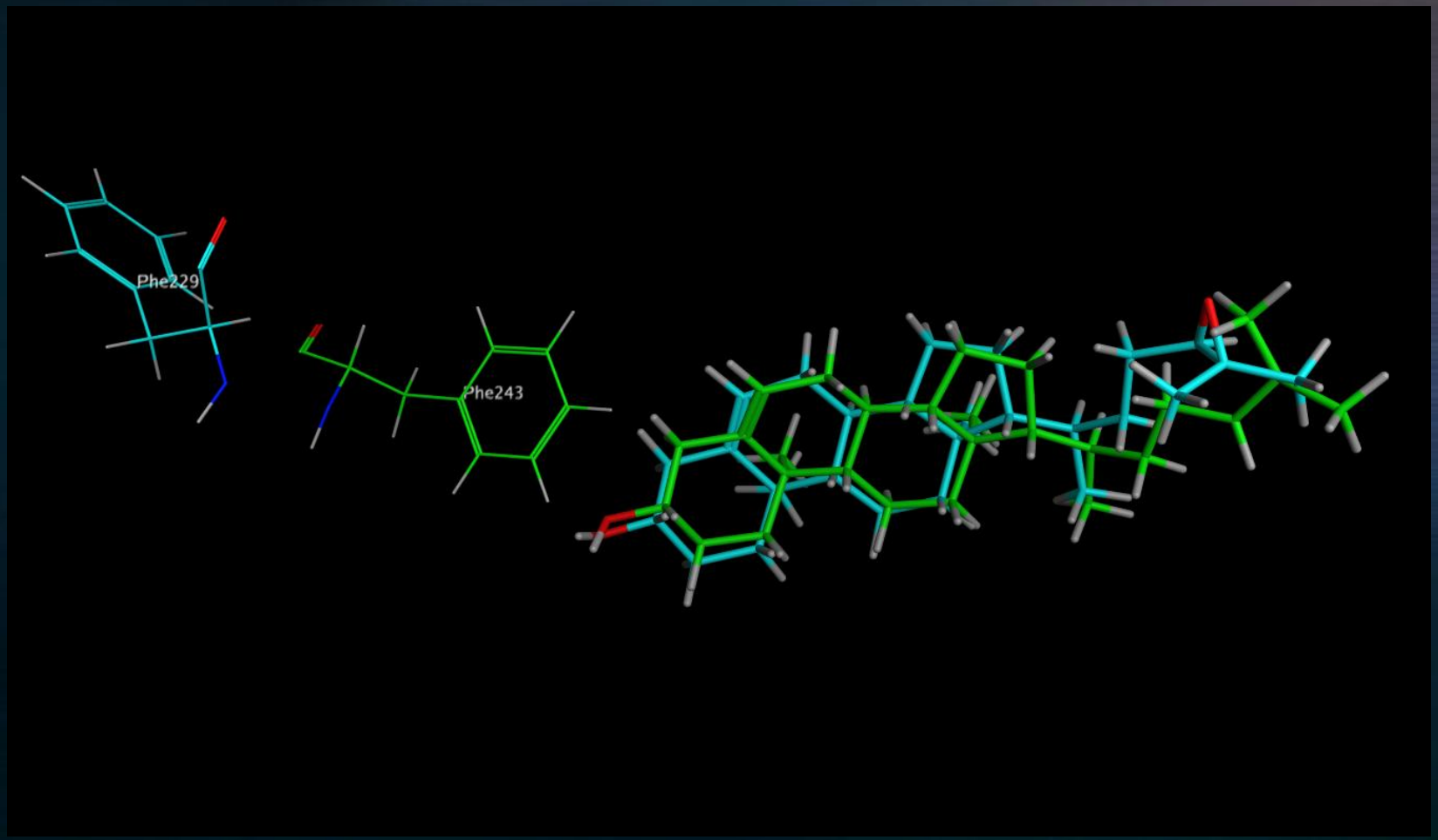


Results


Glycine Scan for DEC
Gln 246
His 435
Ile 309
Ile 353
Phe 243
Phe 340
Wild Type
-10.0
-10.1
-10.2
-10.3
-10.4
-10.5
-10.6
-10.7
-10.8
S-value (kcal/mol)
LXRβ-DEC
LXRα-DEC
Tiny Difference

Results-Glycine Scanning

- Phe 229 (LXRα - cyan) and Phe 243 (LXRβ - green)



Key Findings

- Docking – inconclusive because isoforms have similar binding affinity for DEC
- Dynamics – LXRβ has slightly higher affinity for DEC than LXRα for DEC
  - LXRβ looses affinity over time
- Glycine Scanning – different orientation and binding influence of Phe 229 (LXRα) and Phe 243 (LXRβ)

Future Work

- Optimize parameters for docking
- Run Dynamics for longer periods
- Study LXRβ selective ligands
- Simulate analogs of LXRβ selective ligands
- Study kinetic binding mechanism for complex

Acknowledgements

- Dr. Riley
- LA-SiGMA REU
- NSF
- Xavier University
- Dr. Sridhar
- Sydni Bellow

References

- Viennois, E., et al. Selective liver X receptor modulators (SLiMs): What use in human health? *Mol. Cell. Endocrinol*. **2012**, *351*, 129-141
- Hiranthi, J., et al. Diterpenoid, steroid, and triterpenoid agonists of liver X receptors from diversified terrestrial plants and marine sources. *J. Nat. Prod*. **2005**, *68*, 1247-1252.
- Janowski, B., et al. Structural requitements of ligands for the oxysterol liver X receptors LXRα and LXRβ. *Proc. Natl. Acad. Sci.* **1999**, *96*, 266-271.
- Haung, T., et al. A novel LXR-α activator identified from the natural product *Gynostemma pentaphyllum*. *Biochem. Pharmacol*. **2005**, *70*, 1298-1308.