

Computational Investigation of Isoform Selectivity in Liver X Receptors

Brennan Ferguson

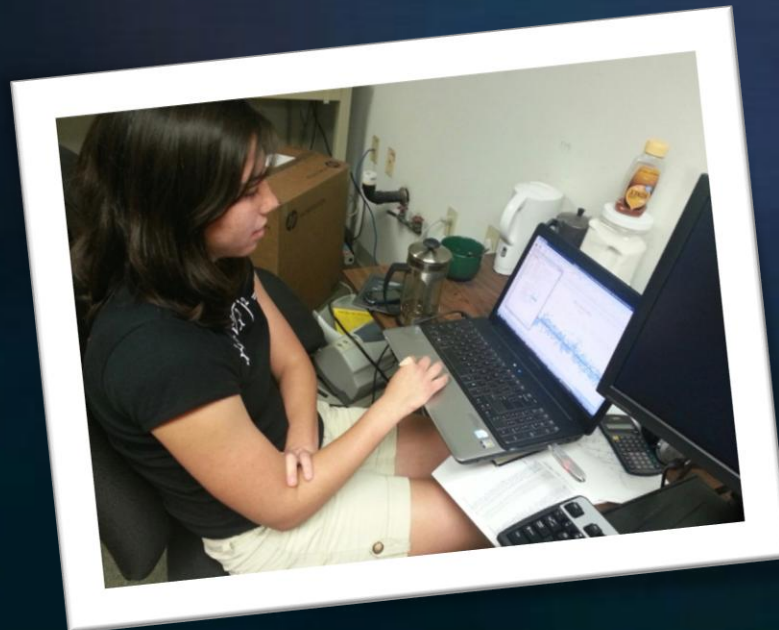
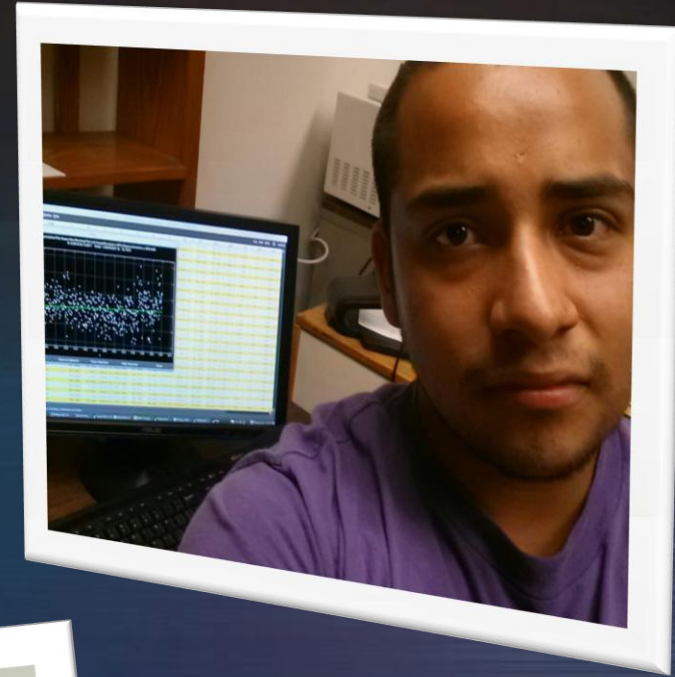
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Presentation Outline

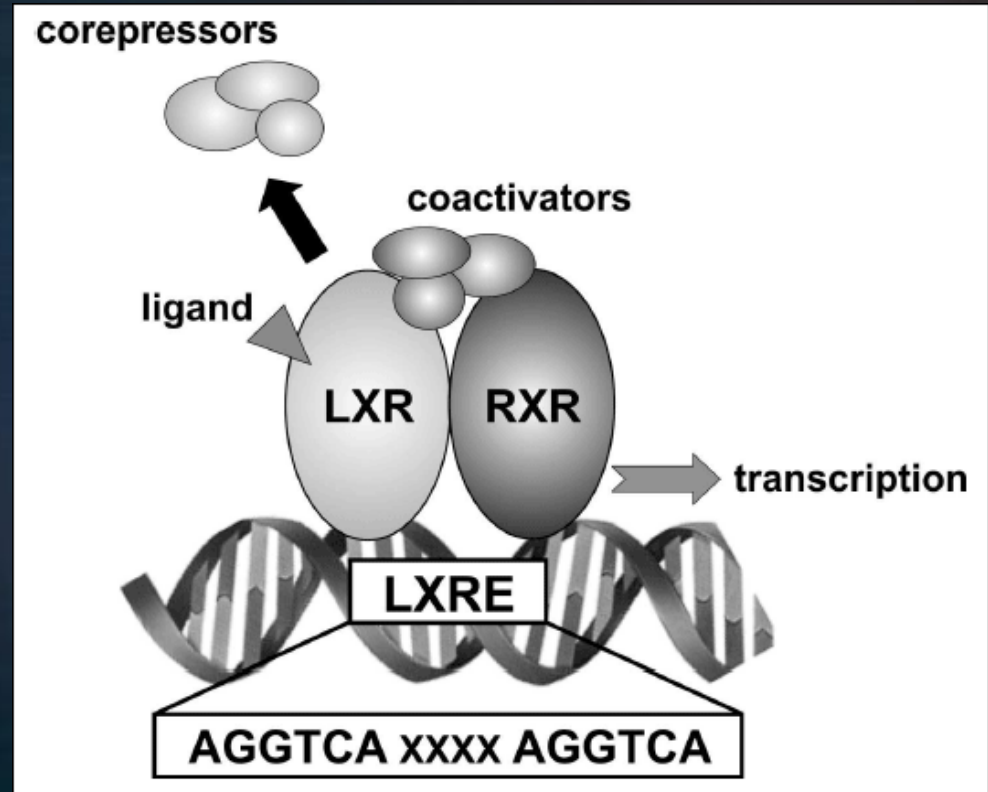
- Introduction
- Theory
- Methods
- Results
- Conclusion



Introduction

● Liver X Receptor

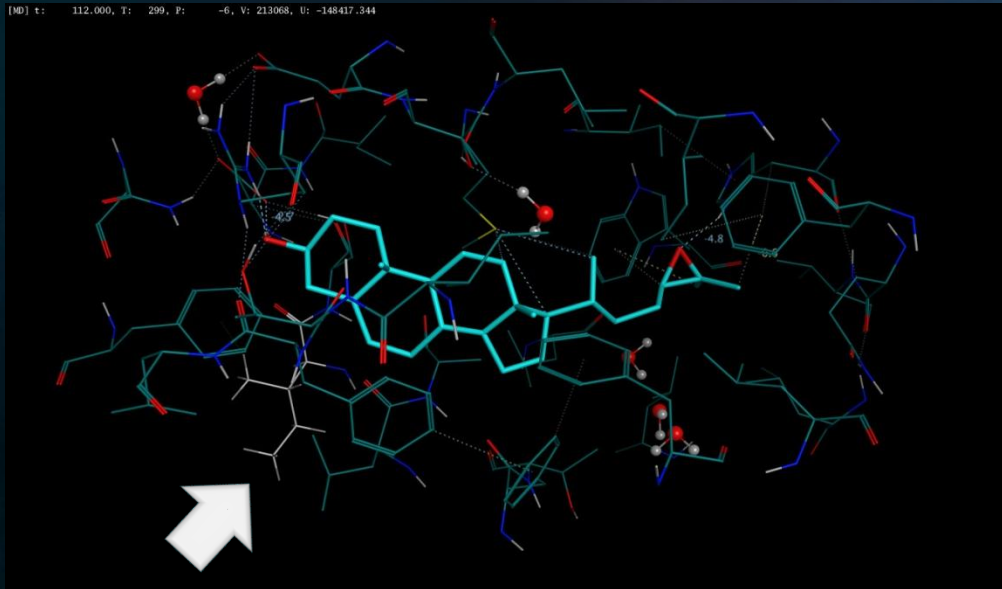
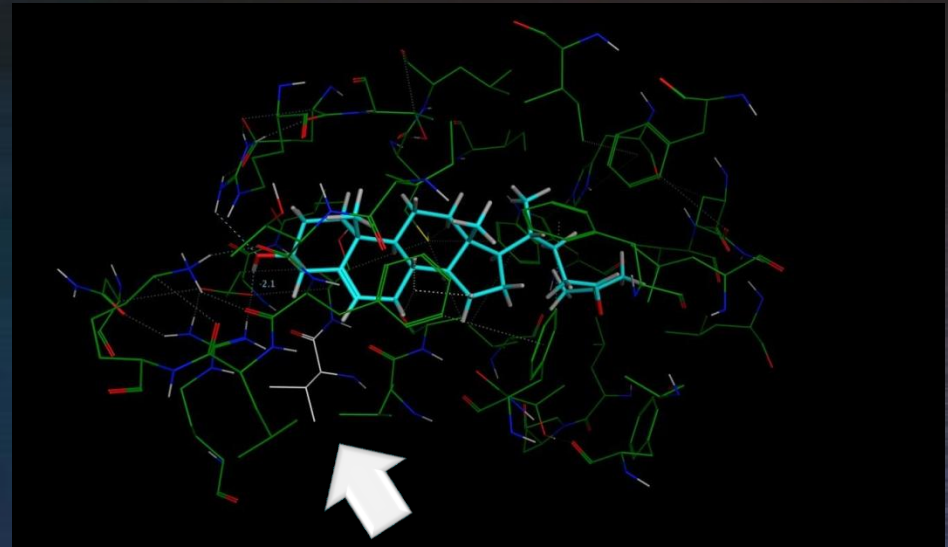
- Nuclear Receptor
- Forms heterodimer with Retinoid X Receptor (RXR)
- α and β isoforms
 - α is found in Liver
 - β is expressed everywhere
- Binds to Cholesterol Derivatives
- Induces Reverse Cholesterol Transport (RCT)



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

Introduction

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



Binding Pocket Difference:
LXR α : Valine
LXR β : Isoleucine

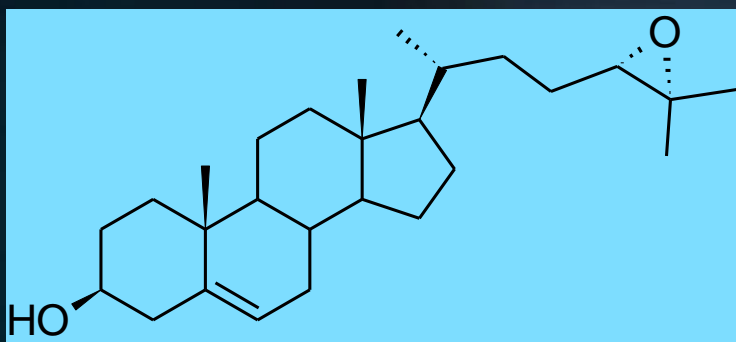
Introduction

+	Tag	Chain	1	5	10	15	20	25	30	35
1P8D	1: 1P8D.B	ACE-VAL-GLN-LEU-THR-ALA-ALA-GLN-GLU-LEU-MET-ILE-GLN-GLN-LEU-VAL-ALA-ALA-GLN-LEU-GLN-CYS-ASN-LYS-ARG-SER-PHE-SER-ASP-GLN-PRO-LYS-VAL-THR-PRO-		220	225	230	235	240	245	250
3IPU	2: 3IPU.A	ACE-PRO-GLN-LEU-SER-PRO-GLU-GLN-LEU-GLY-MET-ILE-GLU-LYS-LEU-VAL-ALA-ALA-GLN-GLN-GLN-CYS-ASN-ARG-ARG-SER-PHE-SER-ASP-ARG-LEU-ARG-VAL-THR-PRO-	205	210	215	220	225	230	235	
+	Tag	Chain	36	40	45	50	55	60	65	70
1P8D	1: 1P8D.B	TRP-PRO-LEU-GLY-ALA-ASP-PRO-GLN-SER-ARG-ASP-ALA-ARG-GLN-GLN-ARG-PHE-ALA-HIS-PHE-THR-GLU-LEU-ALA-ILE-ILE-SER-VAL-GLN-GLU-ILE-VAL-ASP-PHE-ALA-		255	260	265	270	275	280	285
3IPU	2: 3IPU.A	TRP-PRO-MET-ALA-PRO-ASP-PRO-HIS-SER-ARG-GLU-ALA-ARG-GLN-GLN-ARG-PHE-ALA-HIS-PHE-THR-GLU-LEU-ALA-ILE-VAL-SER-VAL-GLN-GLU-ILE-VAL-ASP-PHE-ALA-	240	245	250	255	260	265	270	
+	Tag	Chain	71	75	80	85	90	95	100	105
1P8D	1: 1P8D.B	LYS-GLN-VAL-PRO-GLY-PHE-LEU-GLN-LEU-GLY-ARG-GLU-ASP-GLN-ILE-ALA-LEU-LEU-LYS-ALA-SER-THR-ILE-GLU-ILE-MET-LEU-LEU-GLU-THR-ALA-ARG-ARG-TYR-ASN-		290	295	300	305	310	315	320
3IPU	2: 3IPU.A	LYS-GLN-LEU-PRO-GLY-PHE-LEU-GLN-LEU-SER-ARG-GLU-ASP-GLN-ILE-ALA-LEU-LEU-LYS-THR-SER-ALA-ILE-GLU-VAL-MET-LEU-LEU-GLU-THR-SER-ARG-ARG-TYR-ASN-	275	280	285	290	295	300	305	
+	Tag	Chain	106	110	115	120	125	130	135	140
1P8D	1: 1P8D.B	HIS-GLU-THR-GLU-CYS-ILE-THR-PHE-LEU-LYS-ASP-PHE-THR-TYR-SER-LYS-ASP-ASP-PHE-HIS-ARG-ALA-GLY-LEU-GLN-VAL-GLU-PHE-ILE-ASN-PRO-ILE-PHE-GLU-PHE-		325	330	335	340	345	350	355
3IPU	2: 3IPU.A	PRO-GLY-SER-GLU-SER-ILE-THR-PHE-LEU-LYS-ASP-PHE-SER-TYR-ASN-ARG-GLU-ASP-PHE-ALA-LYS-ALA-GLY-LEU-GLN-VAL-GLU-PHE-ILE-ASN-PRO-ILE-PHE-GLU-PHE-	310	315	320	325	330	335	340	
+	Tag	Chain	141	145	150	155	160	165	170	175
1P8D	1: 1P8D.B	SER-ARG-ALA-MET-ARG-ARG-LEU-GLY-LEU-ASP-ASP-ALA-GLU-TYR-ALA-LEU-LEU-ILE-ALA-ILE-ASN-ILE-PHE-SER-ALA-ASP-ARG-PRO-ASN-VAL-GLN-GLU-PRO-GLY-ARG-		360	365	370	375	380	385	390
3IPU	2: 3IPU.A	SER-ARG-ALA-MET-ASN-GLU-LEU-GLN-LEU-ASN-ASP-ALA-GLU-PHE-ALA-LEU-LEU-ILE-ALA-ILE-SER-ILE-PHE-SER-ALA-ASP-ARG-PRO-ASN-VAL-GLN-ASP-GLN-LEU-GLN-	345	350	355	360	365	370	375	
+	Tag	Chain	176	180	185	190	195	200	205	210
1P8D	1: 1P8D.B	VAL-GLU-ALA-LEU-GLN-GLN-PRO-TYR-VAL-GLU-ALA-LEU-LEU-SER-TYR-THR-ARG-ILE-LYS-ARG-PRO-GLN-ASP-GLN-LEU-ARG-PHE-PRO-ARG-MET-LEU-MET-LYS-LEU-VAL-		395	400	405	410	415	420	425
3IPU	2: 3IPU.A	VAL-GLU-ARG-LEU-GLN-HIS-THR-TYR-VAL-GLU-ALA-LEU-HIS-ALA-TYR-VAL-SER-ILE-HIS-HIS-PRO-HIS-ASP-ARG-LEU-MET-PHE-PRO-ARG-MET-LEU-MET-LYS-LEU-VAL-	380	385	390	395	400	405	410	
+	Tag	Chain	211	215	220	225	230	235	240	244
1P8D	1: 1P8D.B	SER-LEU-ARG-THR-LEU-SER-SER-VAL-HIS-SER-GLU-GLN-VAL-PHE-ALA-LEU-ARG-LEU-GLN-ASP-LYS-LYS-LEU-PRO-PRO-LEU-LEU-SER-GLU-ILE-TRP-ASP-VAL-NME		430	435	440	445	450	455	460
3IPU	2: 3IPU.A	SER-LEU-ARG-THR-LEU-SER-SER-VAL-HIS-SER-GLU-GLN-VAL-PHE-ALA-LEU-ARG-LEU-GLN-ASP-LYS-LYS-LEU-PRO-PRO-LEU-LEU-SER-GLU-ILE-TRP-ASP-VAL-NME	415	420	425	430	435	440	445	

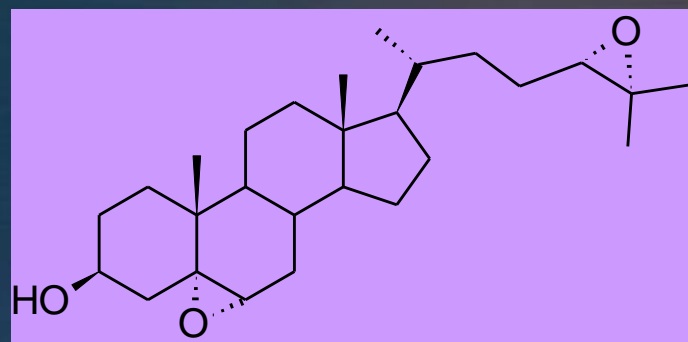
Introduction

- Goal: Investigate Ligand Selectivity

24(s),25-EpoxyCholesterol (EC)



VS



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

- DEC is LXR α selective.

Introduction

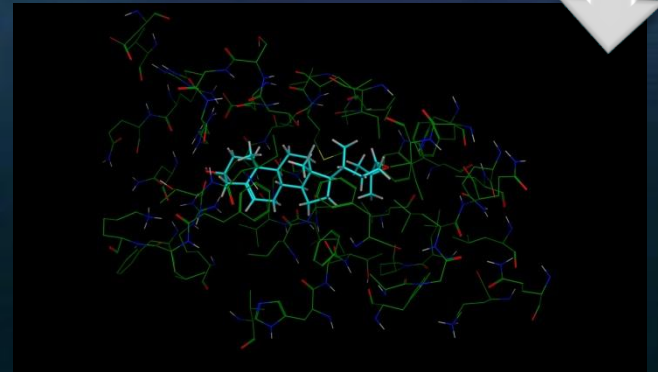
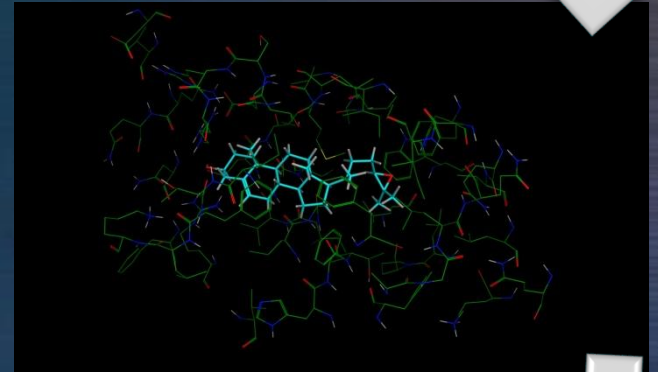
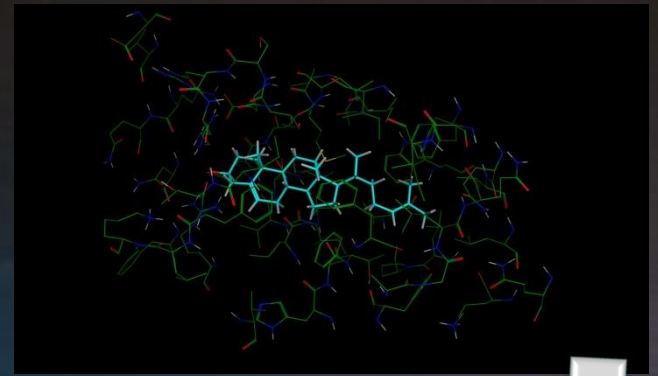
- Question: What determines selectivity?
 - Protein Geometry or Ligand Interaction
- Computational investigations using Molecular Operating Environment (MOE)
 - Docking
 - Molecular Dynamics
 - Glycine Scanning

Introduction:

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

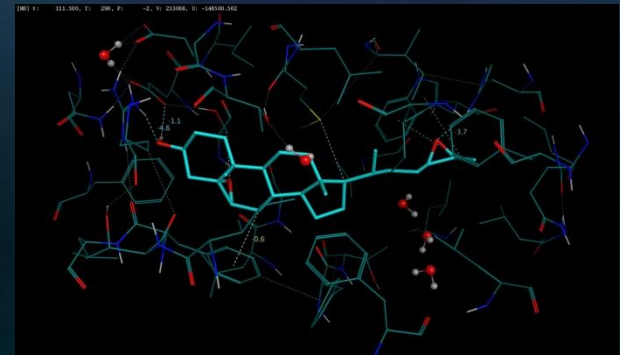
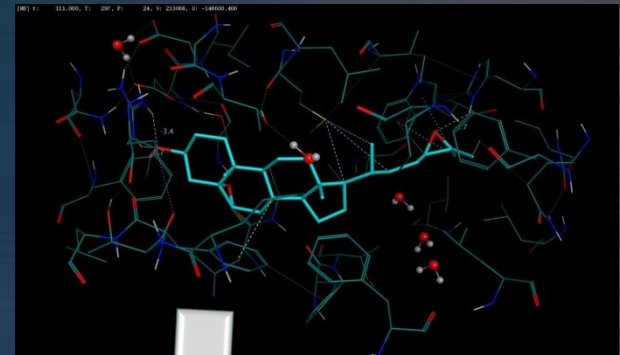
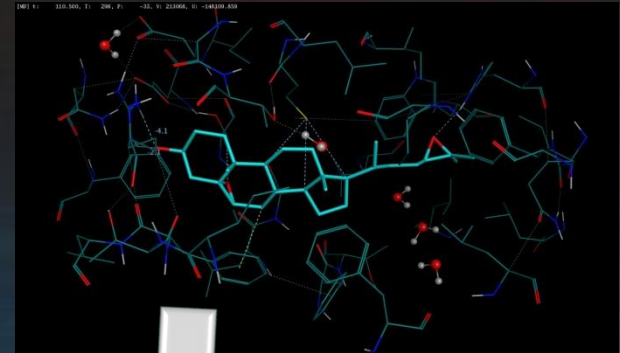
Introduction

- 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

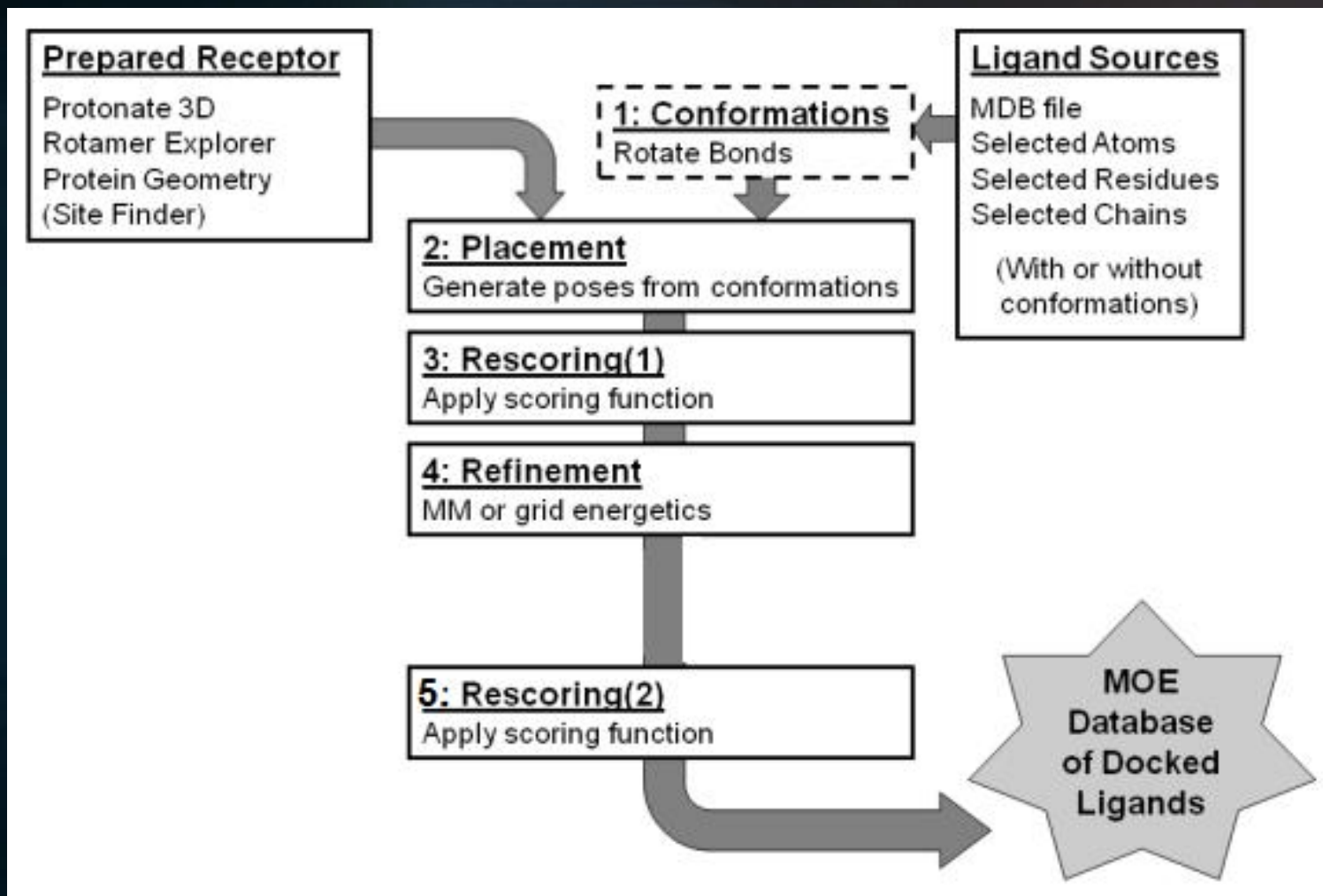


Introduction

- 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

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

- 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

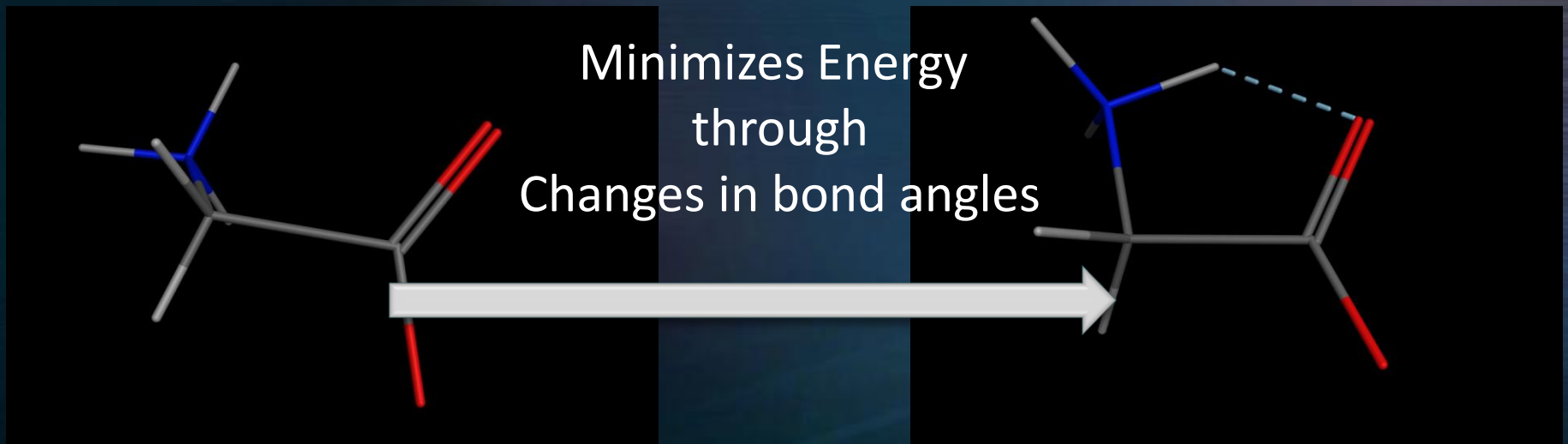
- Estimates from given poses

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

- 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

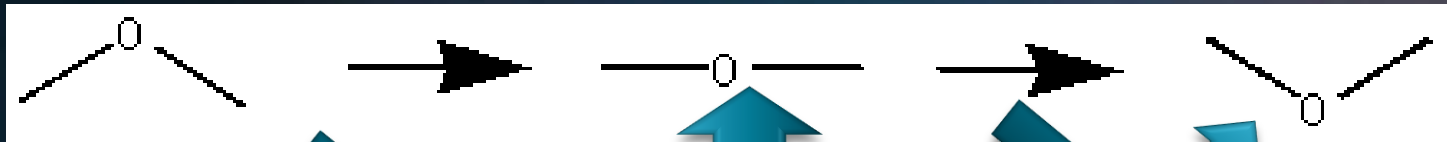
- 3. Refinement
 - Relaxes Strain in System



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

Theory: Molecular Dynamics

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

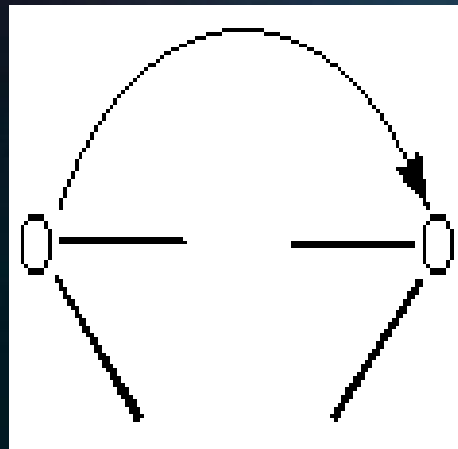


Multiple Changes
In Bond Angle

Highly
Strained

Relieves
Strain

Third Dimension
Holds Energy
Still Rotates

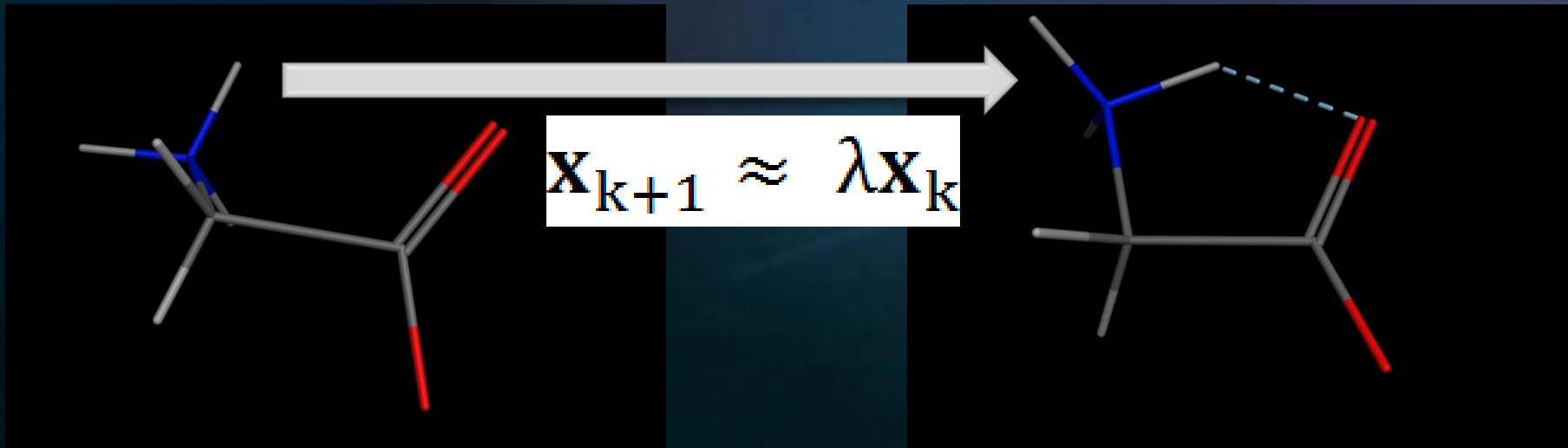


Theory

- 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

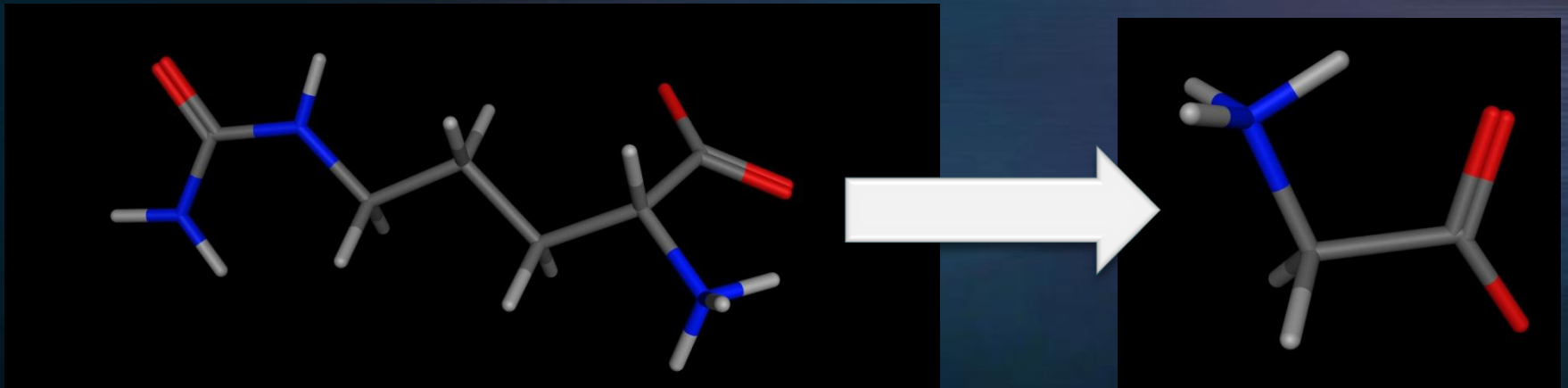
- Molecular Dynamics

$$H(t) = s[\tilde{H}(t) - \tilde{H}(0)]$$
$$\tilde{H}(t) = \frac{\mathbf{u} \cdot \mathbf{M}^{-1} \mathbf{u}}{2s^2V^{2/3}} + \frac{u^2}{2Q_T} + \frac{v^2}{2Q_P} + U(V^{1/3} \mathbf{q}) + gkT \log s + PV$$

- 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)

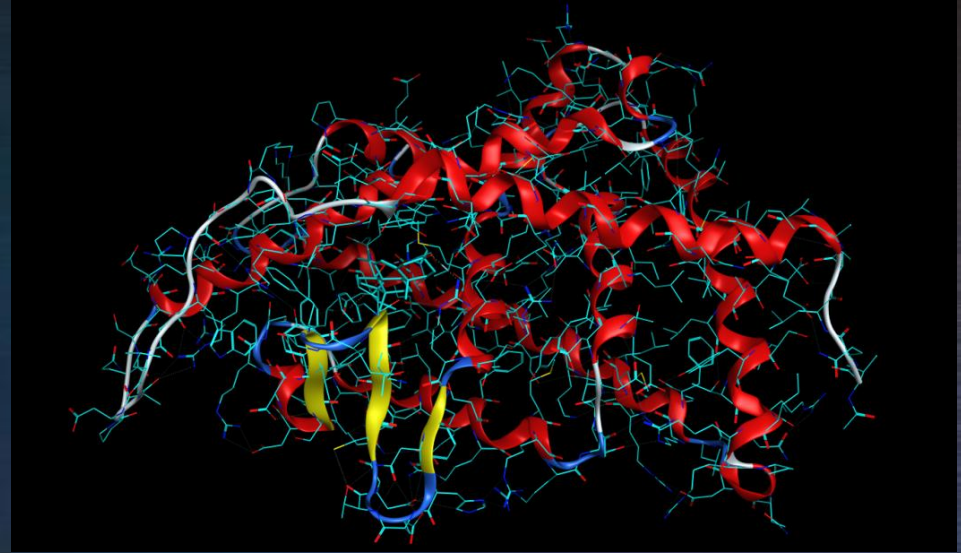


- Re-Score the Mutated Protein

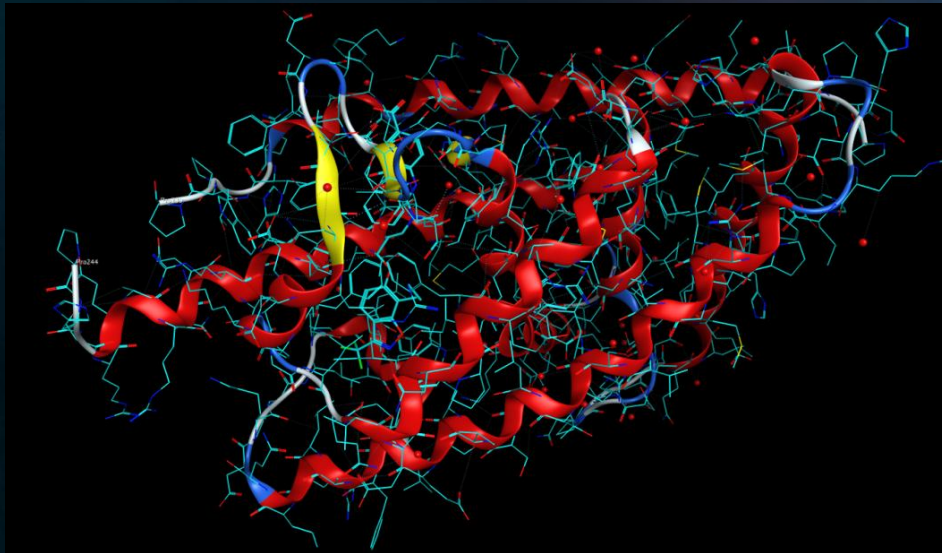
Methods

- Obtained Crystal Structures from Protein Data Bank

IP8D - LXR β



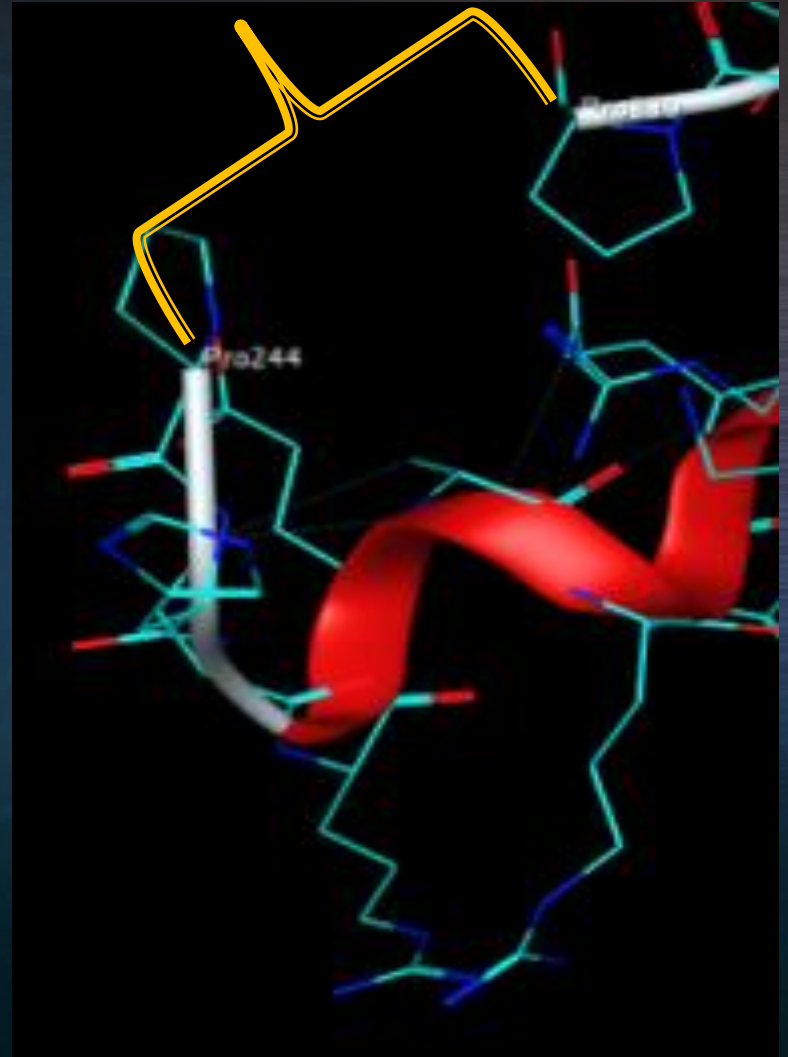
3IPU - LXR α



Methods:

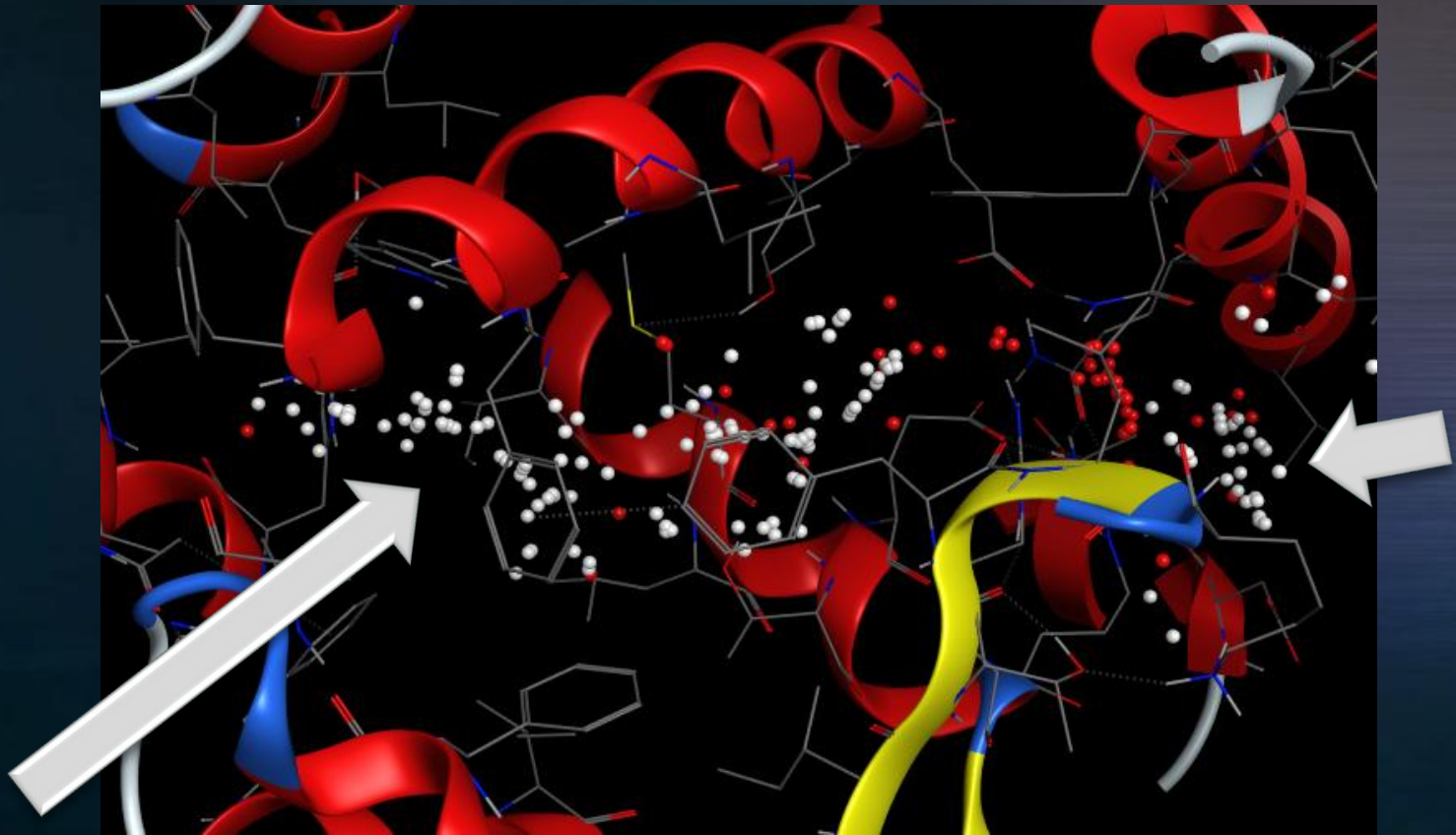
Structure Preparation

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



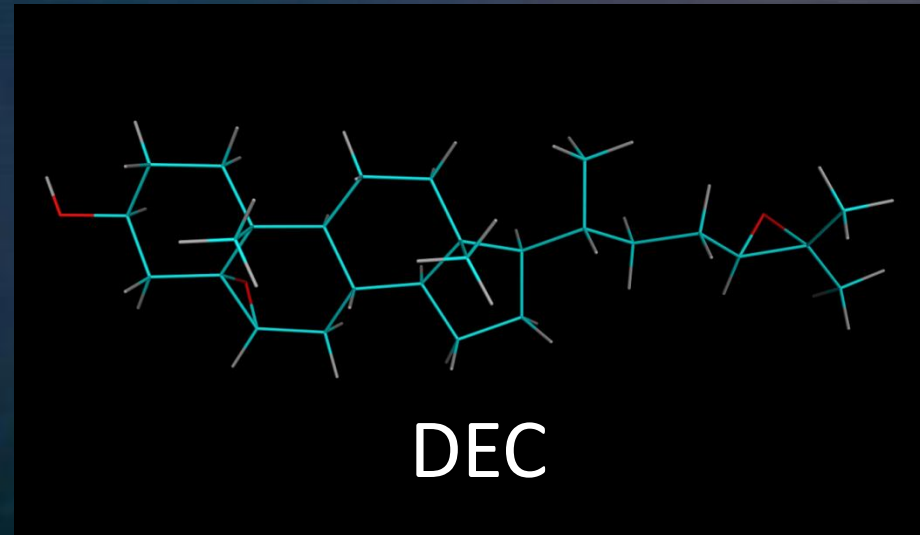
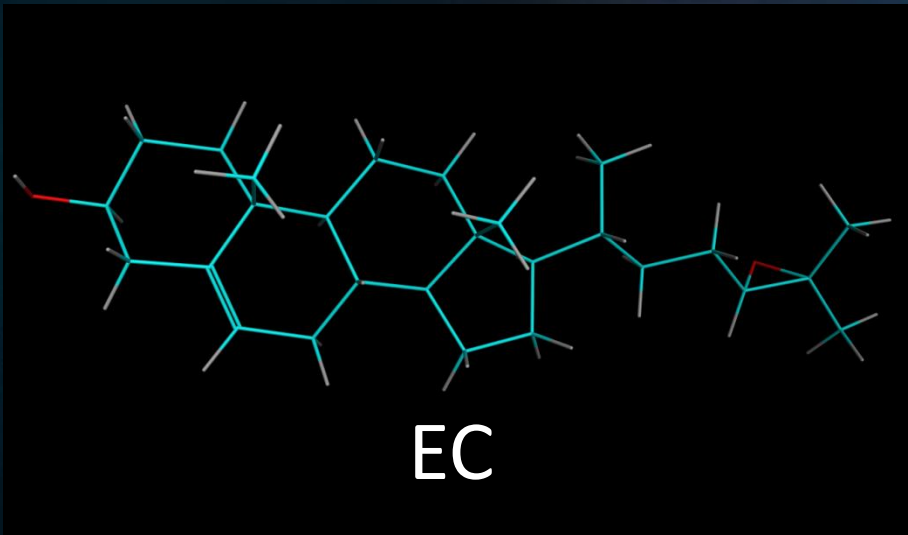
Methods

- Defined the active site



Methods: Ligand Design

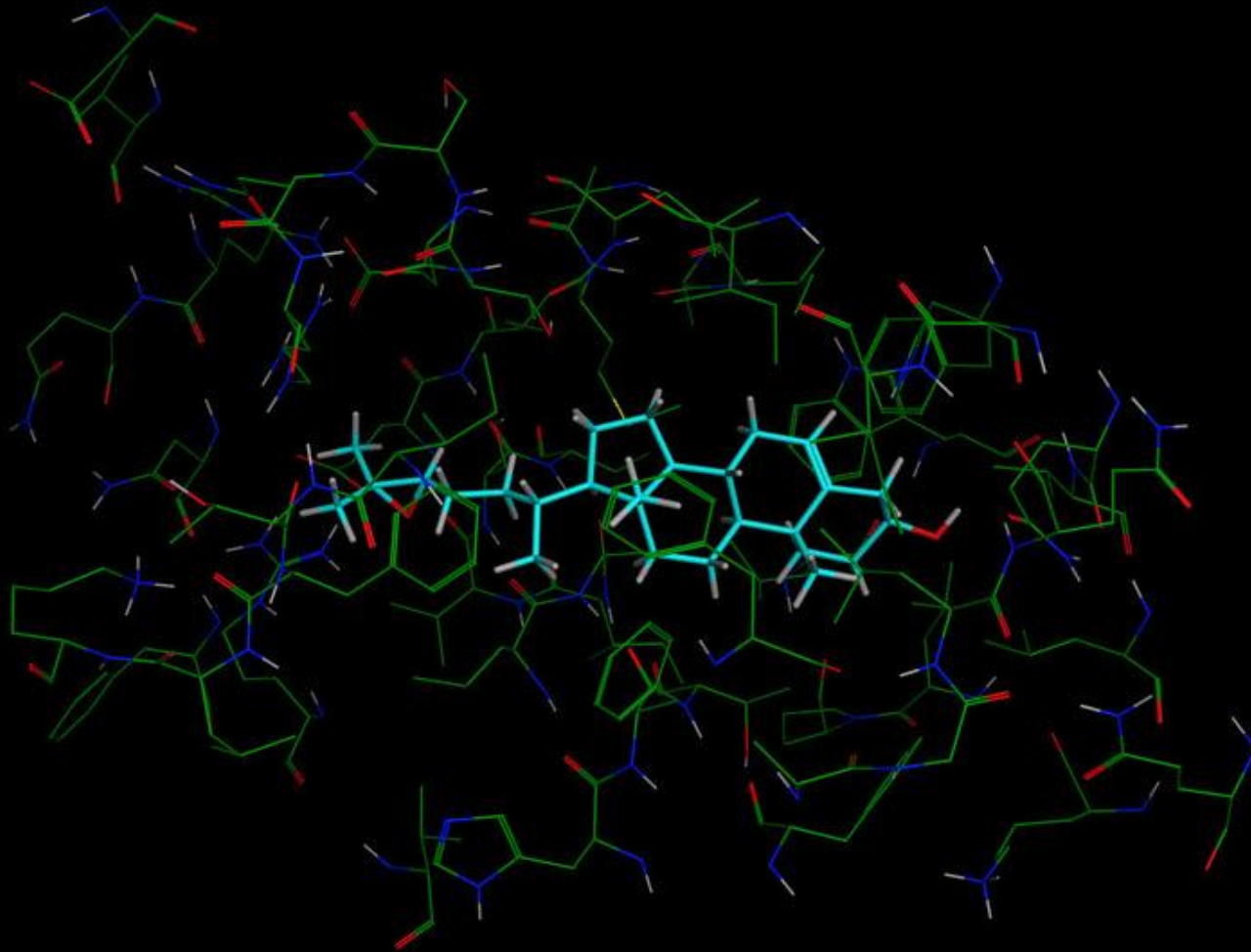
- Built:



Methods: Docking

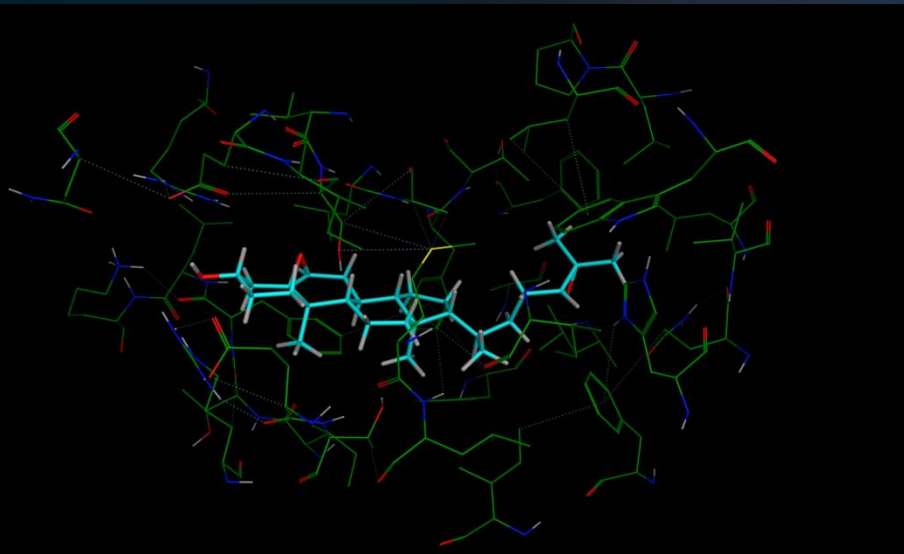
- Used Induced Fit docking to dock EC and DEC to LXR α and LXR β
- Placement: Triangle Matcher
- Rescoring 1: London dG
- Refinement: Force field: AMBER99
- Rescoring 2: GBVI/WSA dG

Methods: Docking

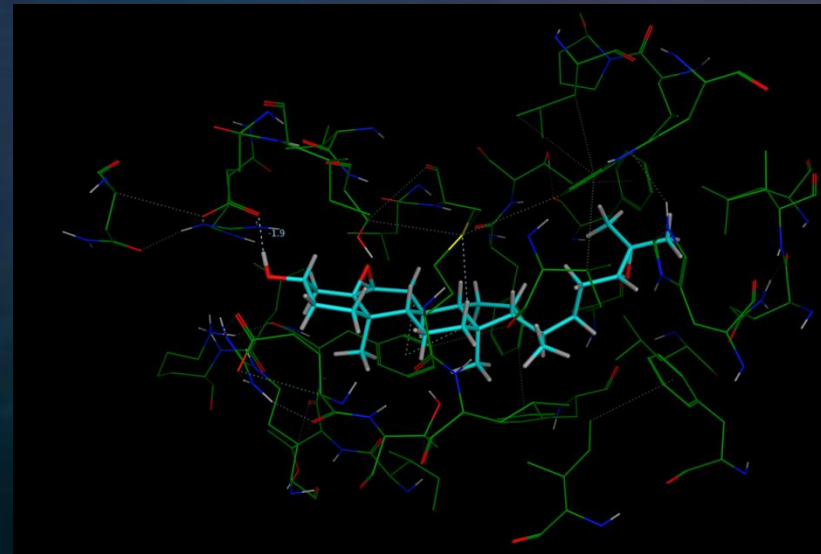


Methods

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



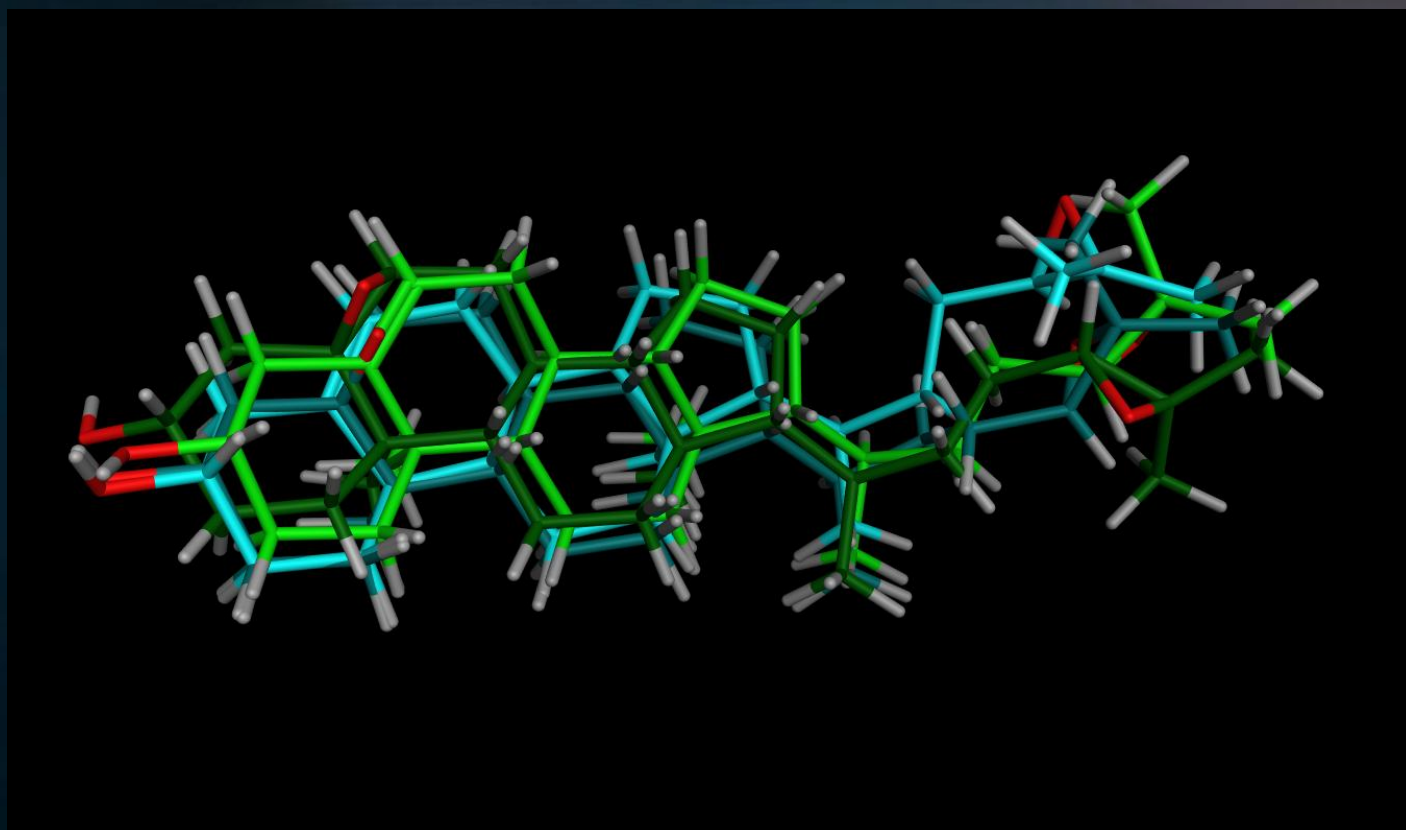
Non-minimized



Minimized

Methods

- Top poses were aligned to compare



Green-1P8D

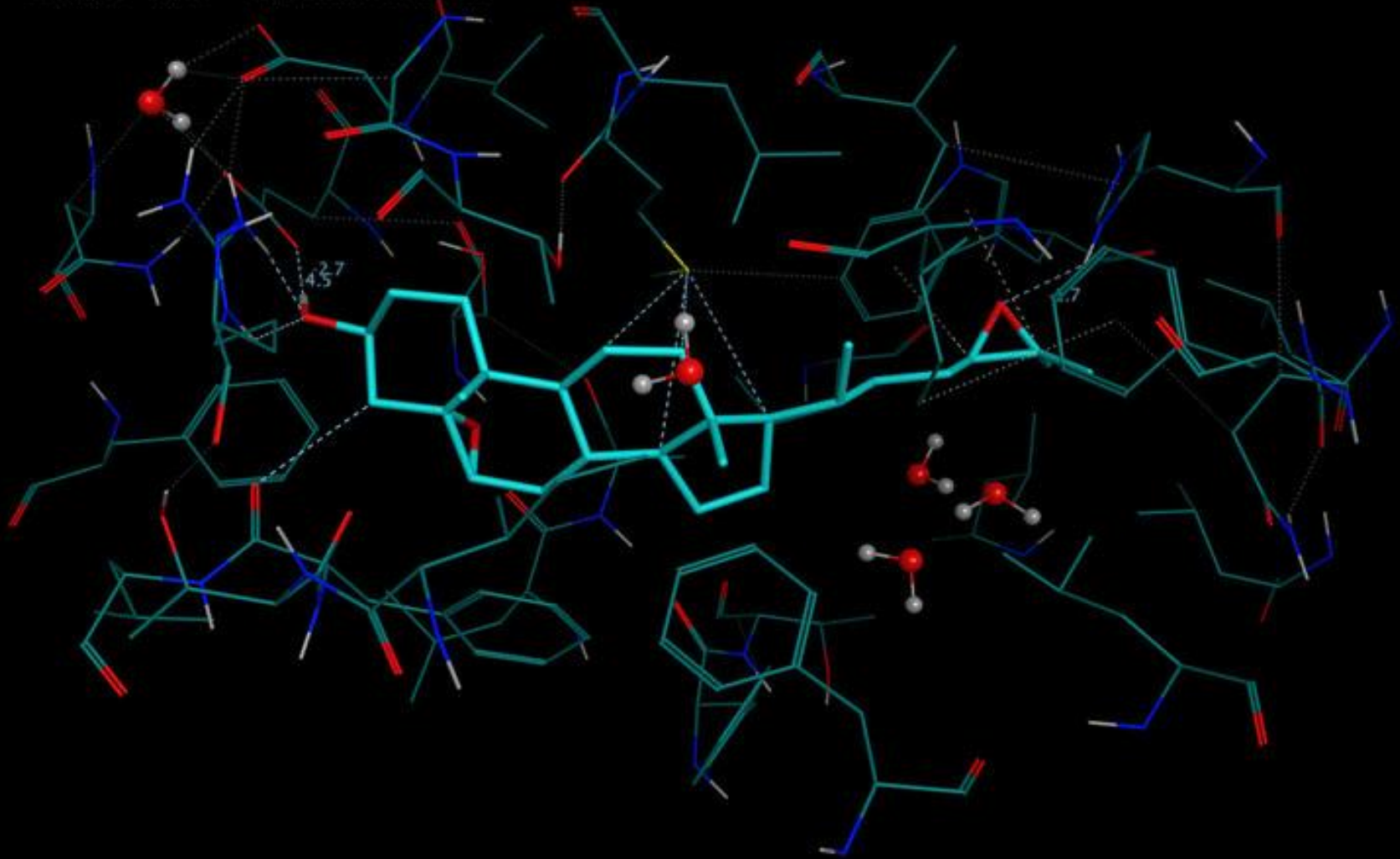
Blue-3IPU

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

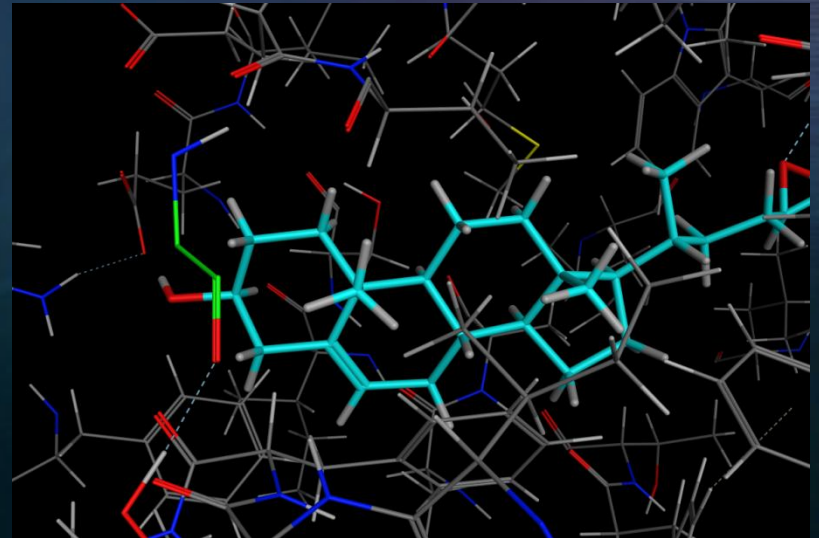
Methods: MD

[MD] t: 100.000, T: 298, P: 24, V: 213068, U: -148260.344



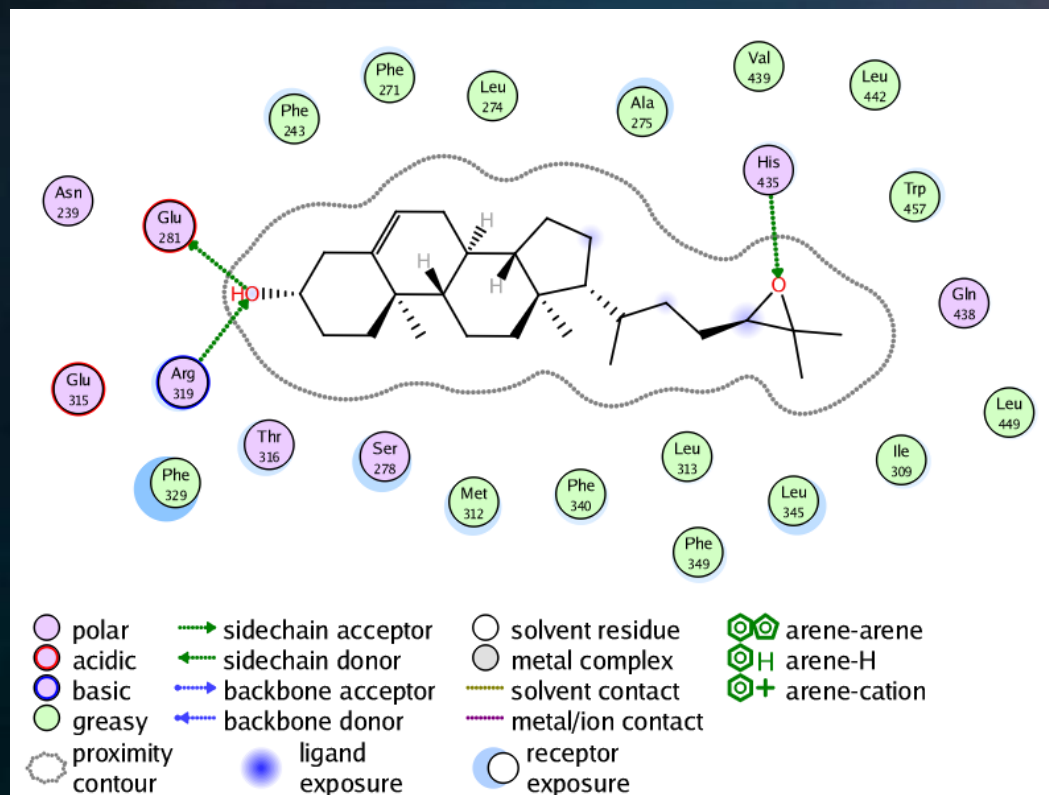
Glycine Scanning

- Glycine scanning for best docked poses and snapshots from dynamics:
 - LXR α – EC
 - LXR α – DEC
 - LXR β – EC
 - LXR β – DEC



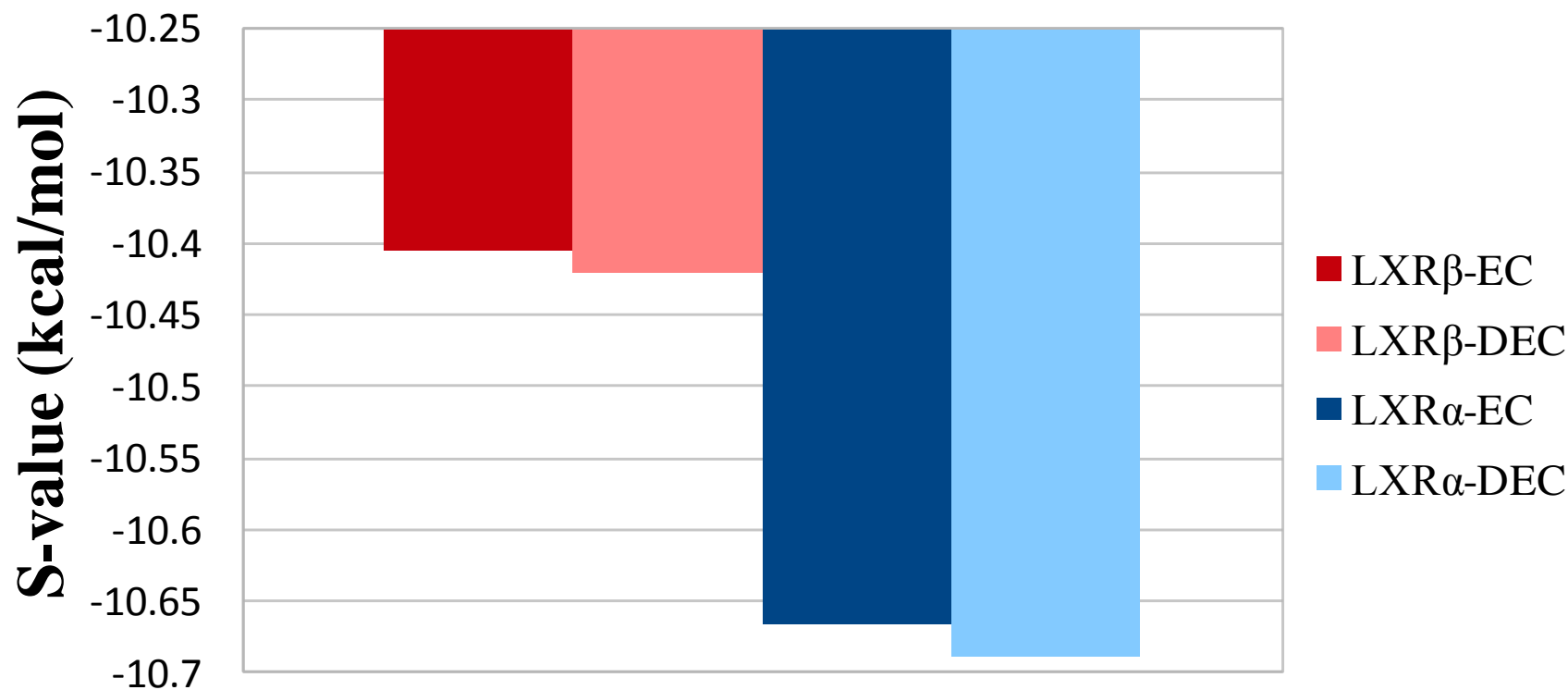
Glycine Scanning

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

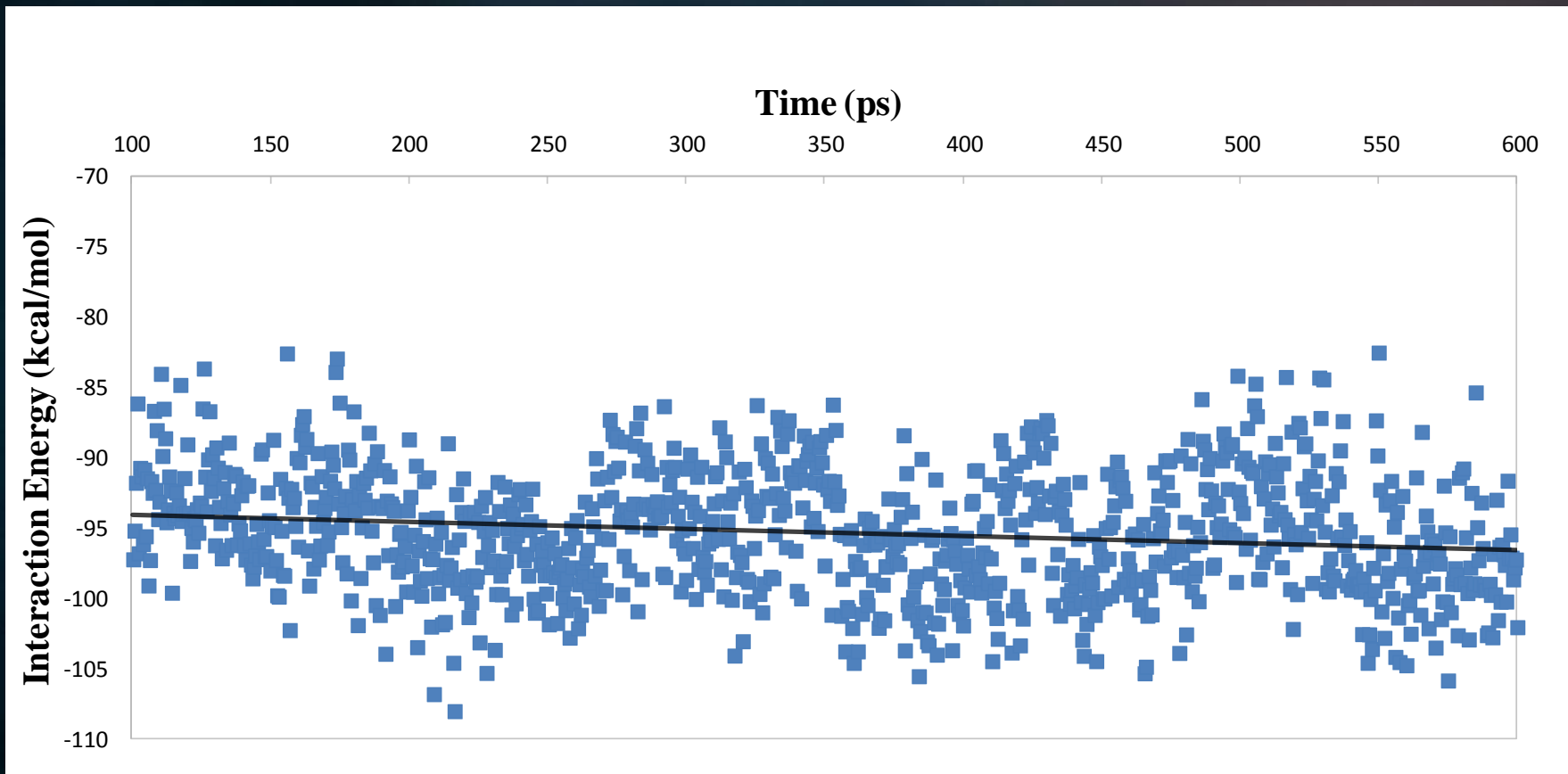


Results - Docking

Docking S-value

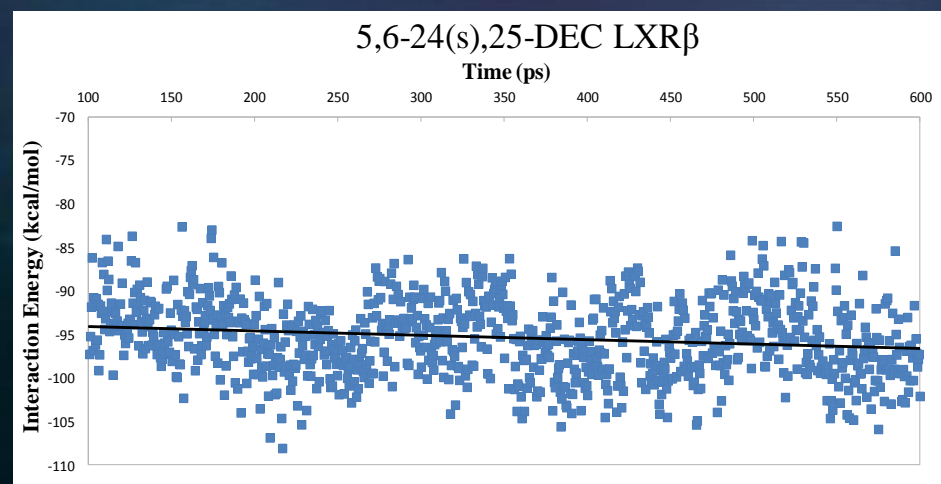
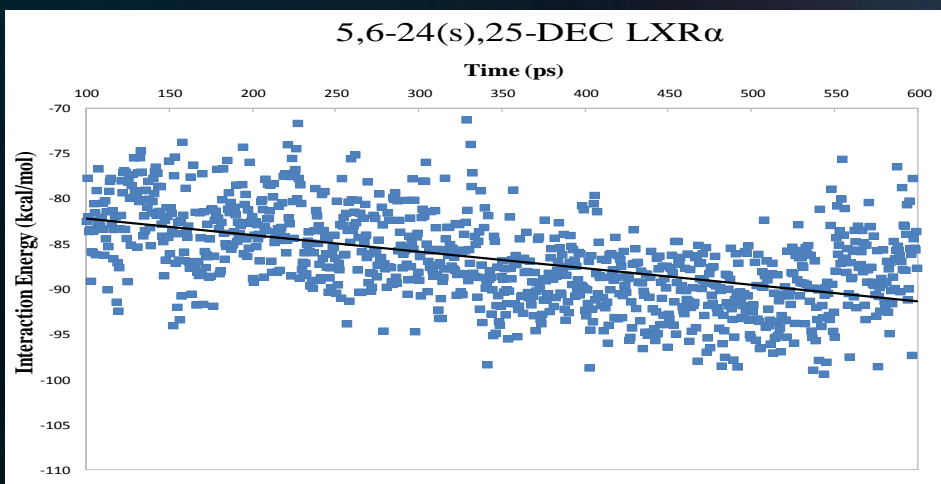
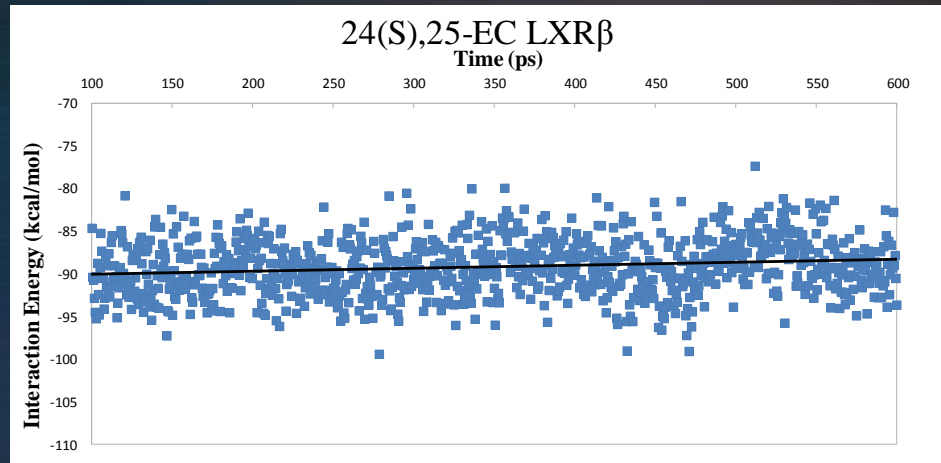
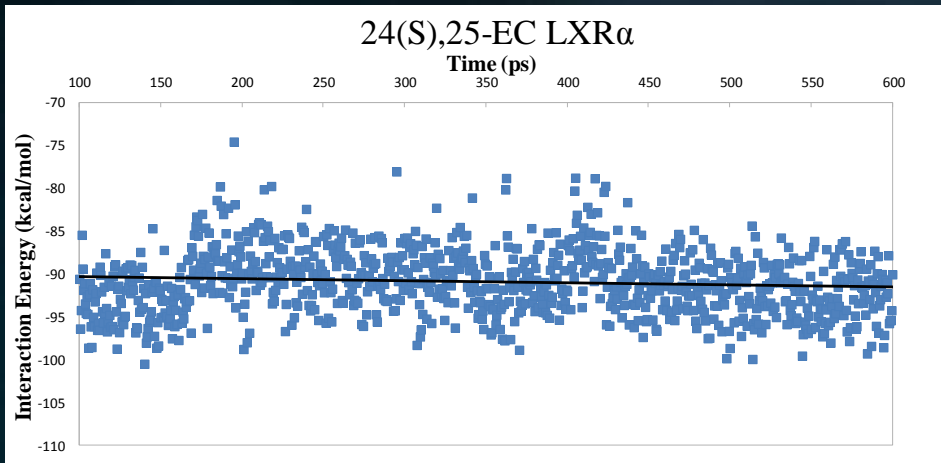


Interaction Energy vs. Time Graph



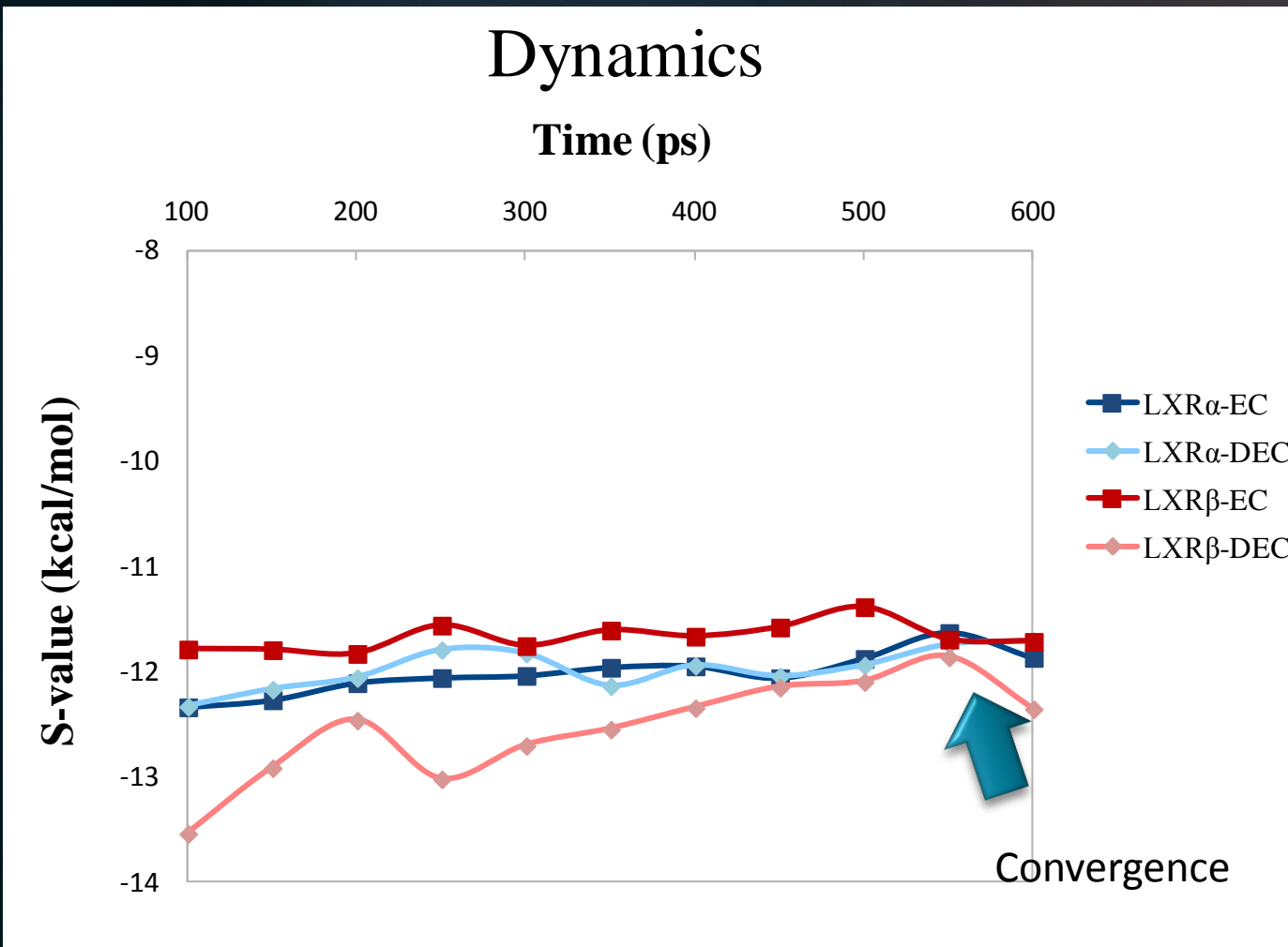
Results - Dynamics

● Potential Interaction Energy



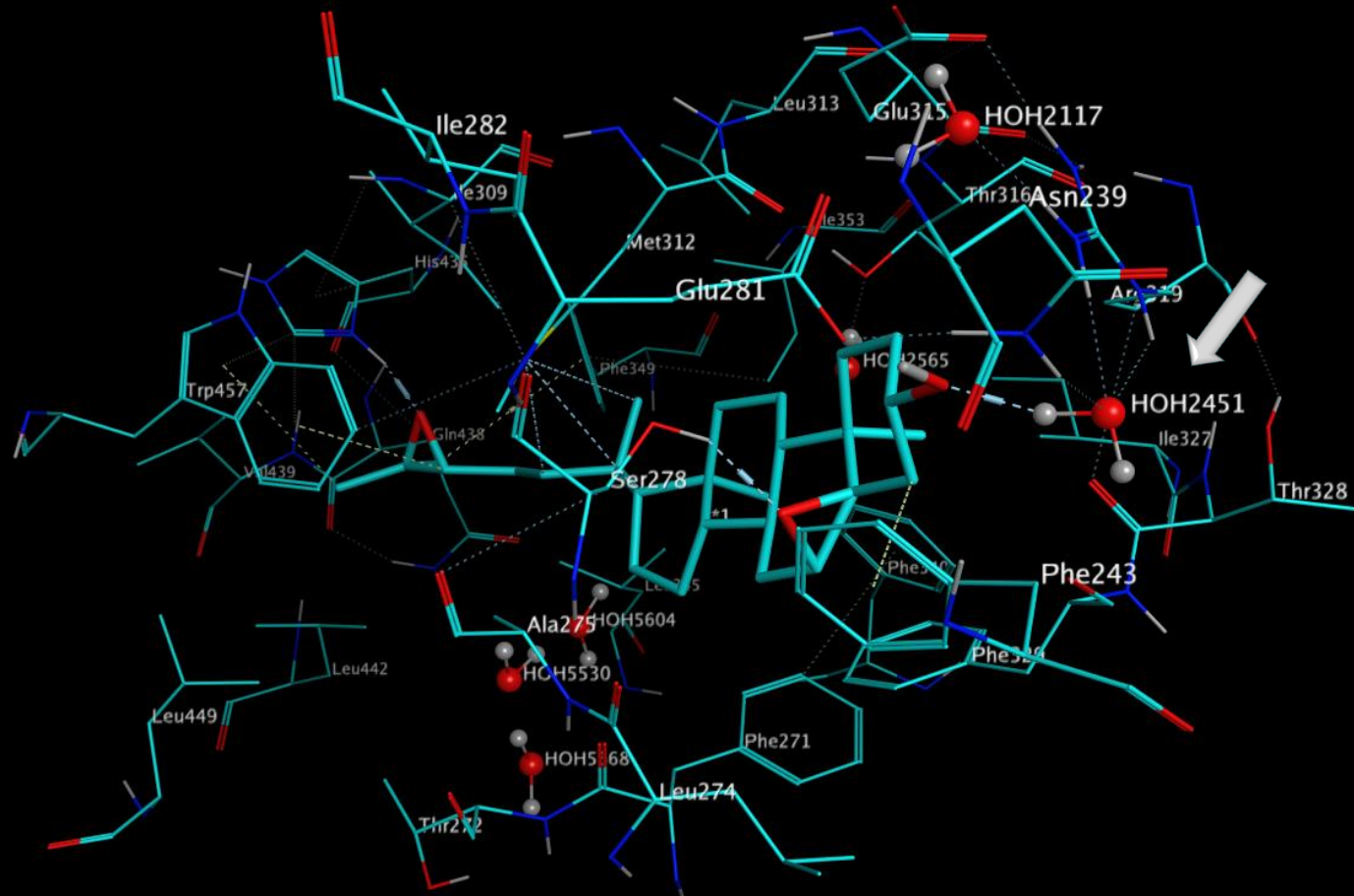
Results-Dynamics

- Snapshot Re-score



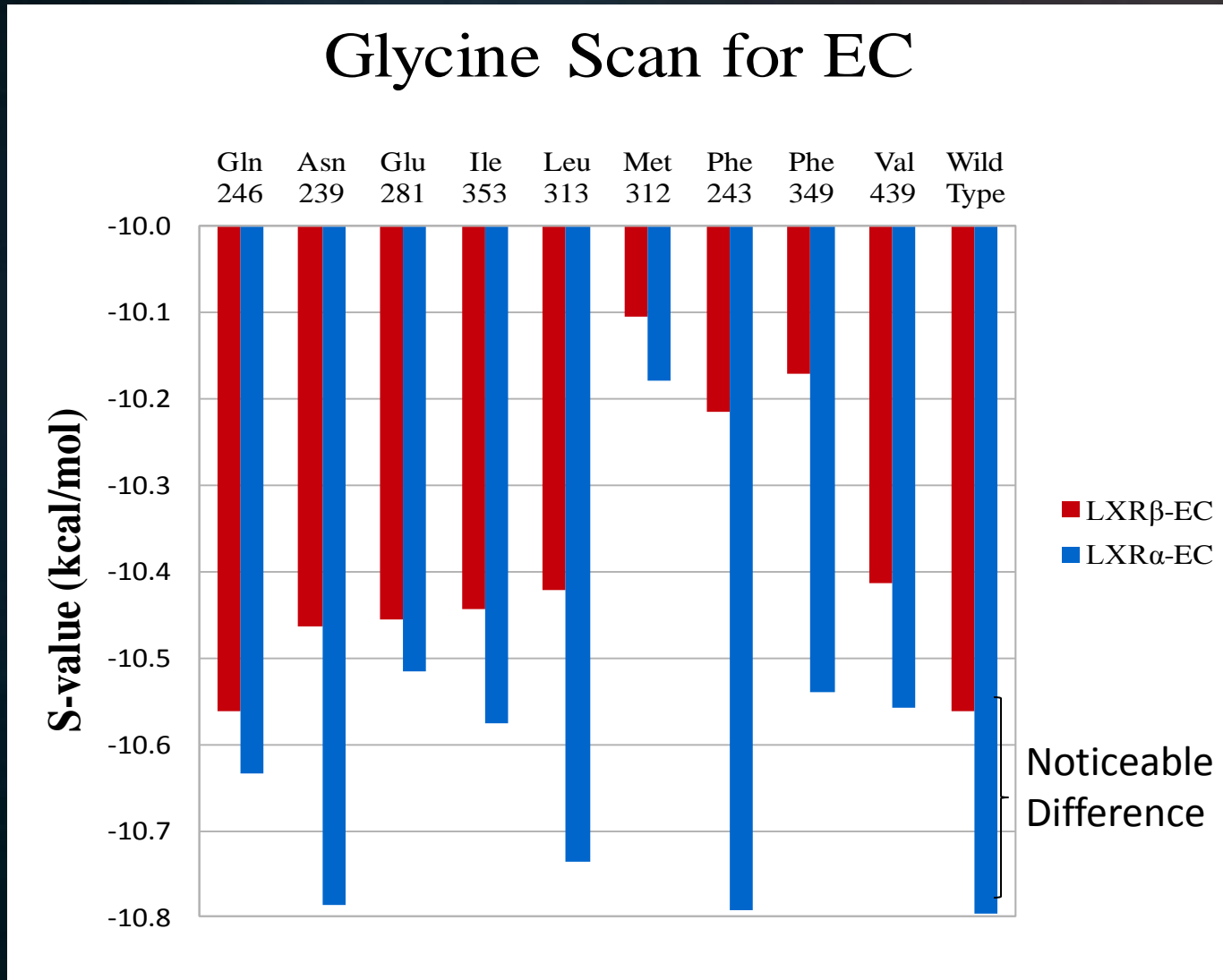
Results - Dynamics

- Web of hydrogen bonds



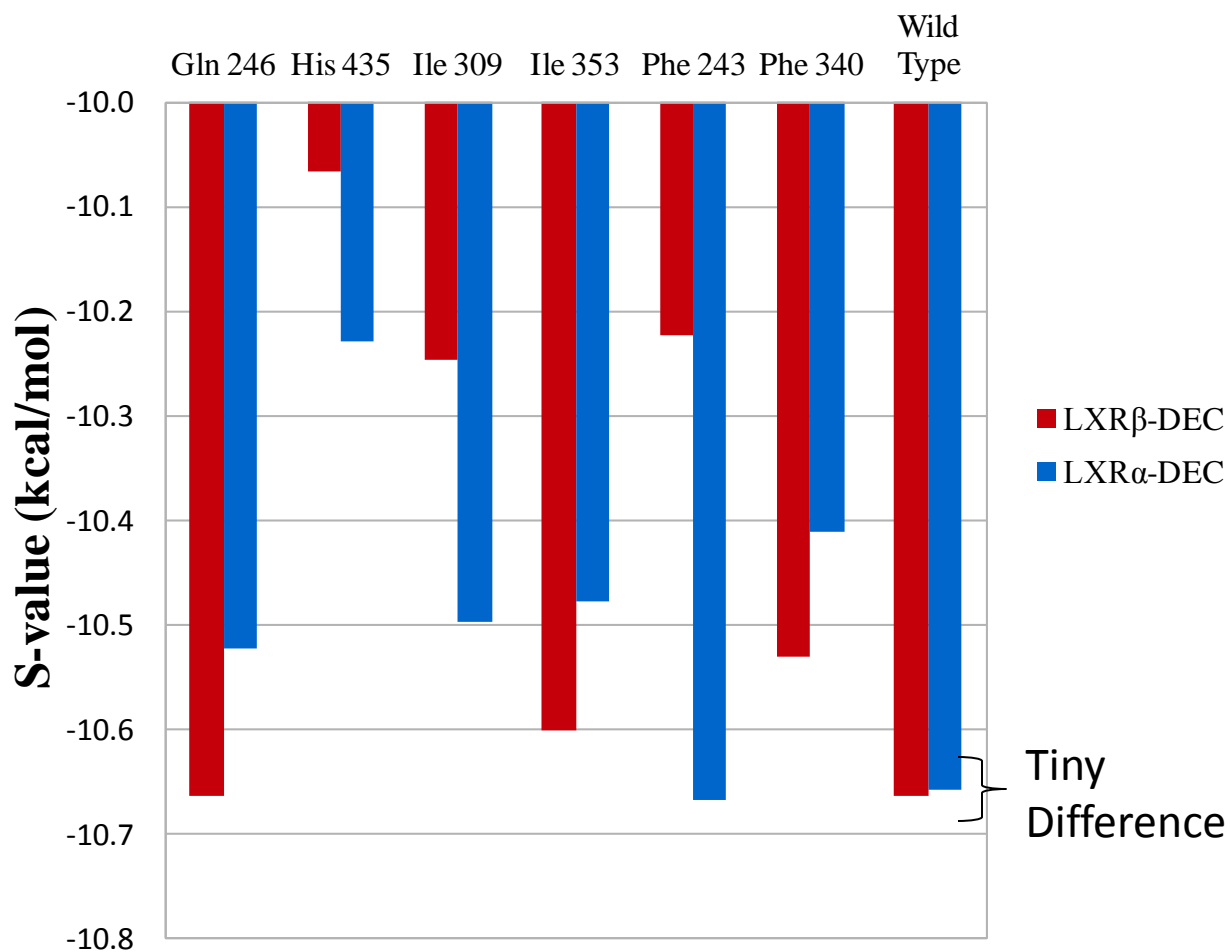
Results-Glycine Scanning

- Significant amino acids



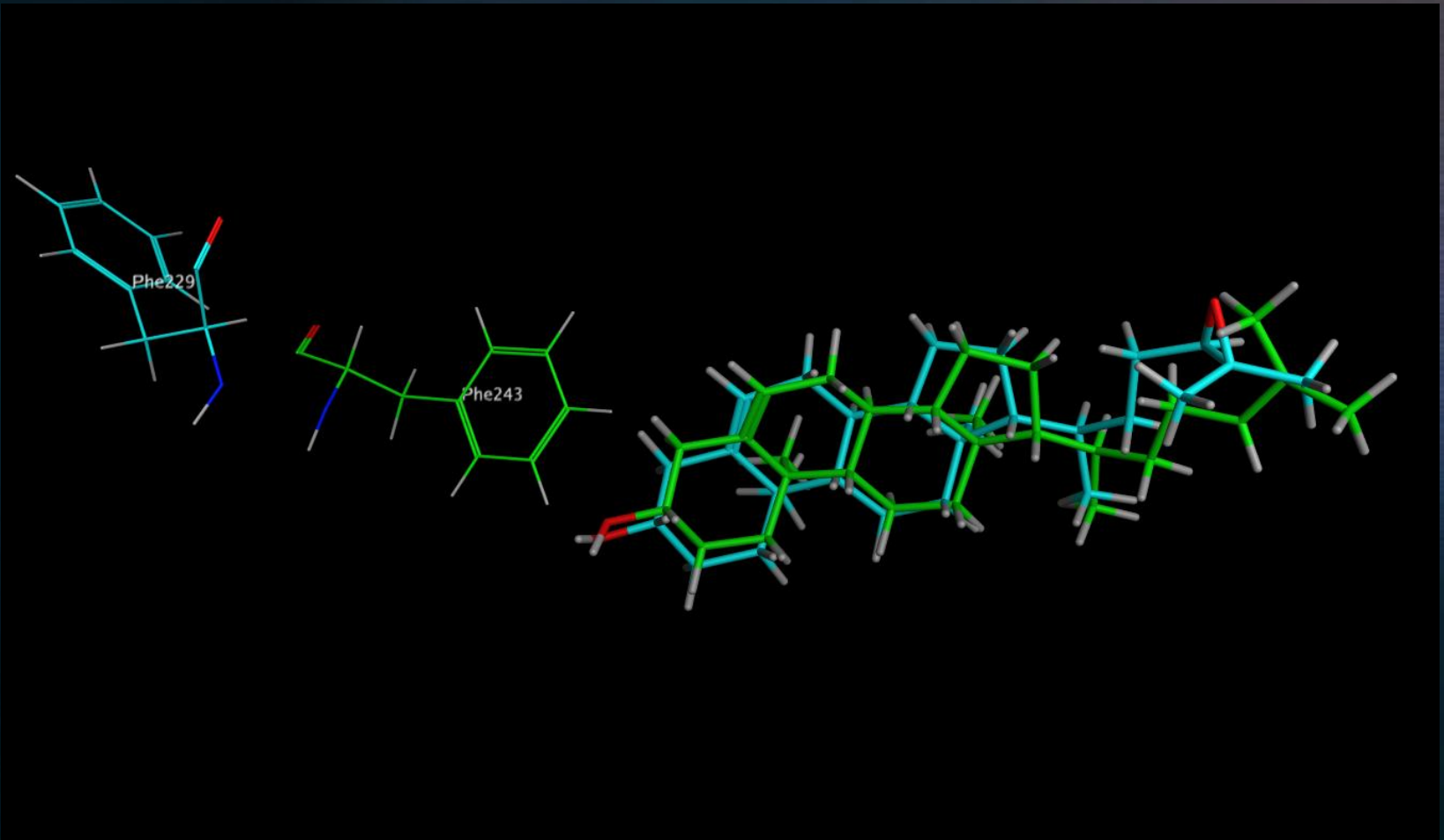
Results

Glycine Scan for DEC



Results-Glycine Scanning

- Phe 229 (LXR α - cyan) and Phe 243 (LXR β - green)



Key Findings

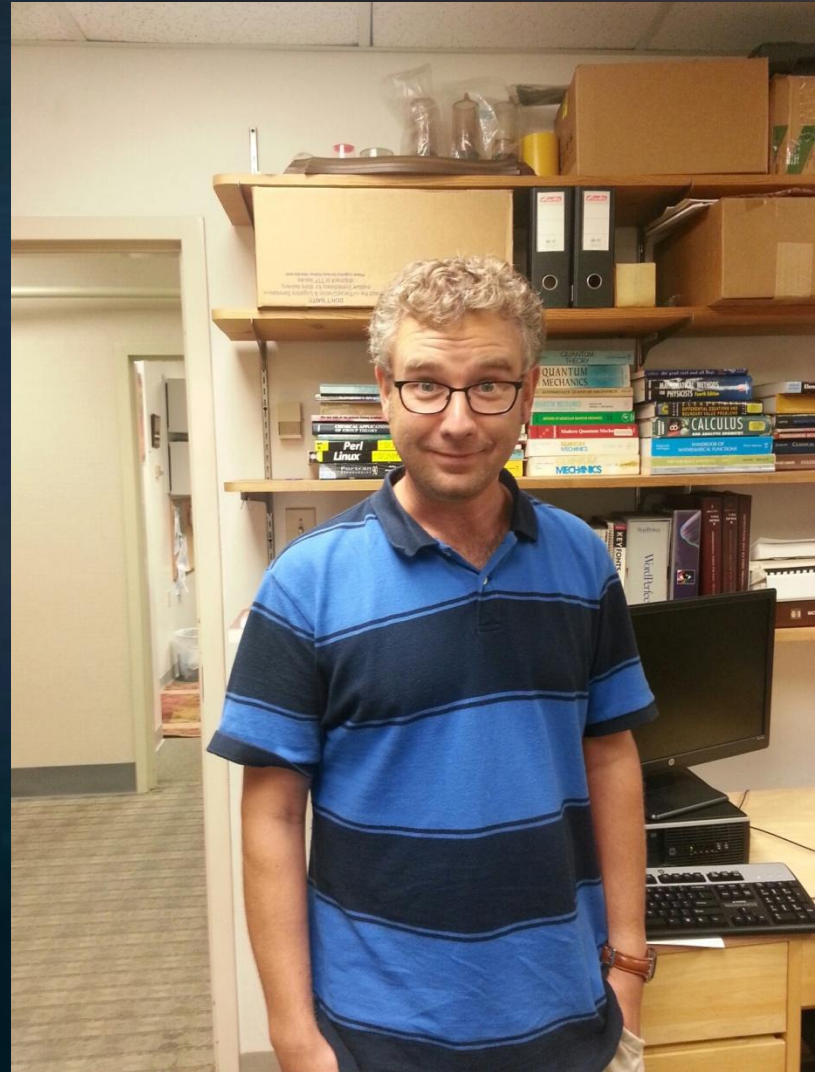
- Docking – inconclusive because isoforms have similar binding affinity for DEC
- Dynamics – LXR β has slightly higher affinity for DEC than LXR α for DEC
 - LXR β loses affinity over time
- Glycine Scanning – different orientation and binding influence of Phe 229 (LXR α) and Phe 243 (LXR β)

Future Work

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

Acknowledgements

- Dr. Riley
- LA-SIGMA REU
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- Dr. Sridhar
- Sydni Bellow



References

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