Finding New Compounds for Soluble Epoxide Hydrolase Inhibition Using Virtual Screening

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Introduction

Soluble Epoxide Hydrolase (sEH) is a protein. Inhibiting sEH with the ligand TPPU has been shown to be an effective pain treatment for neuropathic pain cause by diabetes, specifically in rodents[1].

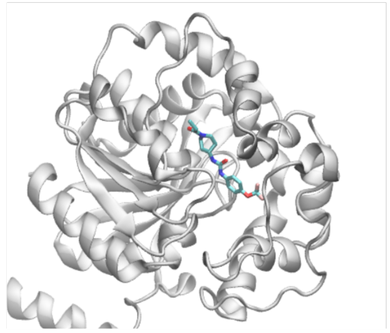


Figure 1: The Crystal Structure of Soluble Epoxide Hydrolase (sEH) and TPPU [PDB ID: 4od0, [1]] - The above figure shows the crystal structure sEH protein in grey and the TPPU ligand in sticks [1]. Picture rendered in VMD [2].

Objective

Identify new structures that could serve as inhibitors of soluble Epoxide Hydrolase, and therefore, possible pain treatments.

Methods

1. Selected Two Poses of Bound sEH and TPPU:
Conformations from a **previous unbinding simulation** of sEH and TPPU were chosen based on the **SASA** (solvent accessible surface area), **RMSD** (root-mean-square deviation), **probability, and interactions** [4]. **Figure 2** depicts the data used from the previous study, and **Figure 3** demonstrates the criteria that was used to select the bound poses, and **Figure 4** is a reference of the interactions

2. Pharmacophore-based Screens:
The selected bound conformations and the crystal structure, were uploaded to **Pharmit** [5] (i.e. online tool used to search a database of structures based on the given structure and pharmacophores).

3. Found Potential Inhibitors:
Ligands that have the **similar features** in the **similar orientation** as the given sEH-TPPU pose were returned as a **ligand dataset**.

4. Selected Two Transition State Poses:
Conformations were chosen based on the **probability**.

5. Docked Ligand Sets on the Protein Conformations:
The crystal structure and the two alternative bound states were docked onto each of the sEH protein poses using Schrödinger Glide [6] docking software.

6. Analyzed Docking Scores:
Found ligands with **low docking scores** (good score) for docking to the bound state conformations as well **high docking scores** (bad score) the transition state conformations. **Figure 5** demonstrates why.

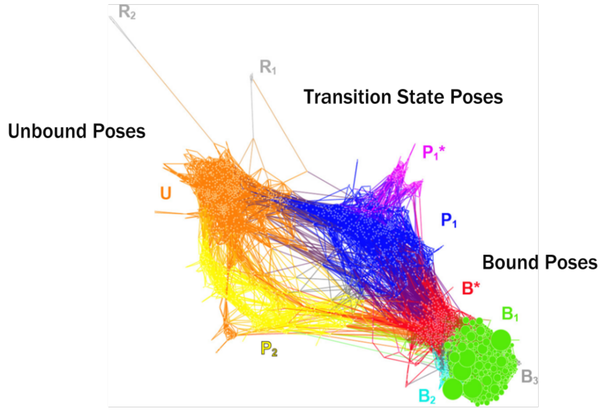


Figure 2: Conformation Space Network of All Poses from a Previous Unbinding Simulations of sEH and TPPU [4] - The graph above is a visual representation of the previous unbinding simulations [4] that poses were selected from. The data from that simulation was clustered and each node is a cluster representative and represents a sEH protein and TPPU ligand conformation. The B nodes are indicate bound poses. P regions represent the transition state conformations, and U regions are unbound conformations. Lastly, the R1 and R2 regions are ligand poses of reversed orientation relative to the native orientation.

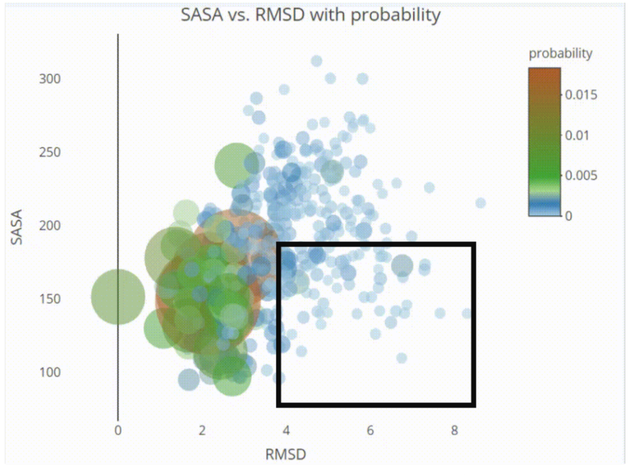


Figure 3: The SASA, RSMD, and Probability of Bound State Poses - The graph above depicts the relationship between the SASA, RMSD, and probability of the cluster representatives in the bound conformation and was used to select the alternative bound state poses that would be screened. The box denotes the set criteria.

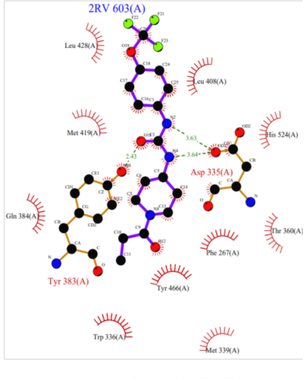


Figure 4: LIGPLOT Diagram of Interactions Ligand 2RV (TPPU) with the Crystal Structure [3] - Hydrogen bonds are denoted by the green dotted line along with the measurement of distance between the atoms in Angstroms (Å), and non-bonded interactions are denoted by the small red lines.

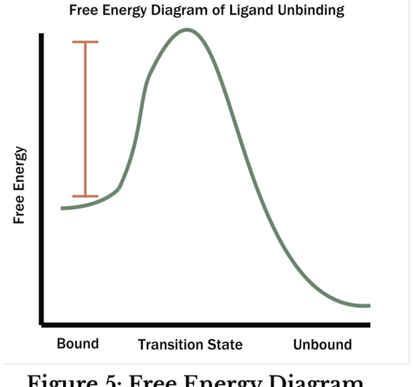
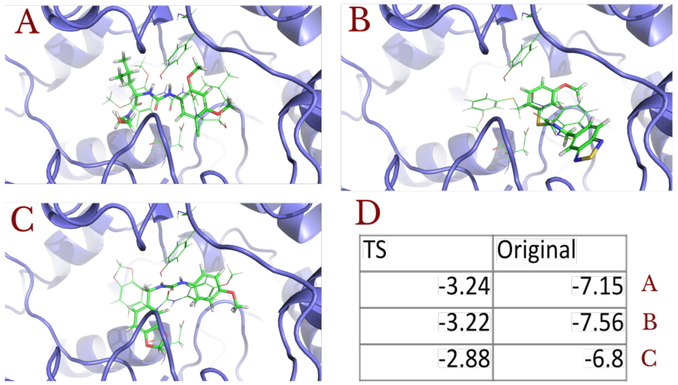


Figure 5: Free Energy Diagram of a Ligand Unbinding from a Protein - The goal is to find ligands that **maximize the free energy difference** between the bound state and the transition state shown in brown.

Results



TS	Original	
-3.24	-7.15	A
-3.22	-7.56	B
-2.88	-6.8	C

Figure 6: Sample Docked Ligand Poses - The docked ligand poses are shown as sticks and lines for the transition state (TS) and bound conformations respectively, and the original protein pose is presented in cartoon style in Figure 6A-C. Figure 6D displays scores for each poses when docked to the transition state (TS) and the original bound crystal structure. Images for 6A-C were rendered in PyMOL [7].

There were **2,422** were **unique structures** out of a total of 12, 131 structures docked with docking scores below -6.5, **287 structures overlapped** for the three bound poses. When compared to the one of the transition state's docking scores, many structures showed a significant difference. The structures A, B, C shown in **Figure 6** are the three structures with the **highest docking scores** (bad score) in the transition state.

Conclusion & Future Work

The structures found in through virtual screening maximize the free energy difference and therefore inhibit sEH for a greater amount of time can be now used in **new unbinding simulations** and **classical molecular dynamics (MD) simulations**. Structures that exhibit long residence times in the unbinding simulations and stability in the binding site for MD simulations could be **potential pain treatments**.

References

1. Lee, Kin Sing Stephen; et. al. 2014. Optimized Inhibitors of Soluble Epoxide Hydrolase Improve in Vitro Target Residence Time and in Vivo Efficacy. Journal of Medicinal Chemistry 2014 *57* (16), 7016-7030.
2. Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38.
3. Laskowski, R. A.; Hutchinson, E. G.; Michie, A. D.; Wallace, A. C.; Jones, M. L.; Thornton, J. M. PDBsum: A Web-Based Database of Summaries and Analyses of All PDB Structures. Trends Biochem. Sci. 1997, 22 (12), 488–490.
4. Lotz, Samuel D and Dickson, Alex. 2018. Unbiased Molecular Dynamics of 11 min Timescale Drug Unbinding Reveals Transition State Stabilizing Interactions. Journal of the American Chemical Society 2018 140 (2), 618-628.
5. Jocelyn Sunseri David Ryan Koes Nucleic Acids Research, Volume 44, Issue W1, 8 July 2016, Pages W442–W448
6. Schrödinger Release 2018-2: Glide, Schrödinger, LLC, New York, NY, 2018.
7. The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

Acknowledgements

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