Unbiased Molecular Dynamics of 11 min Timescale Drug Unbinding Reveals Transition State Stabilizing Residues

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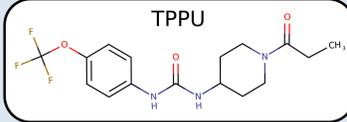Michigan State University, East Lansing, MI

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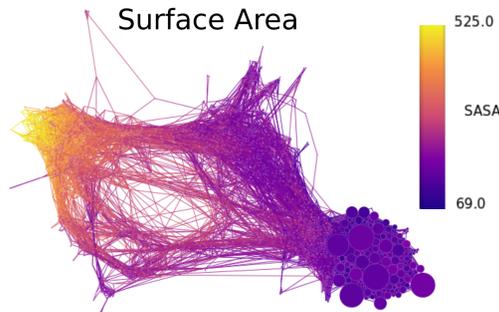linkedin: in/salotz

Introduction

Recent interest in drug binding kinetics (1) has motivated all atom molecular dynamics simulations of ligand (un)binding which has only become feasible due to increases in computational power and improvements in sampling algorithms. We report sampling of the unbinding of a drug-like inhibitor (TPPU) of the protein soluble epoxide hydrolase (sEH) with an experimentally determined residence time of 11 min. We conclude that **specific interactions with residues at the edges of the binding site are important stabilizers of the unbinding transition state.** The specific structural details of these interactions will be of interest to medicinal chemists designing drugs with longer residence times, which has been shown to improve efficacy. sEH is an important target for treating a number of diseases such as diabetic neuropathic pain.

TPPU

Bottleneck Contributions

% Flux / Cumulative % Flux vs Bottleneck

The highest flux pathway passes through P_1. This path's bottleneck contributes 4x more flux than the most paths, though many paths contribute to total network flux.

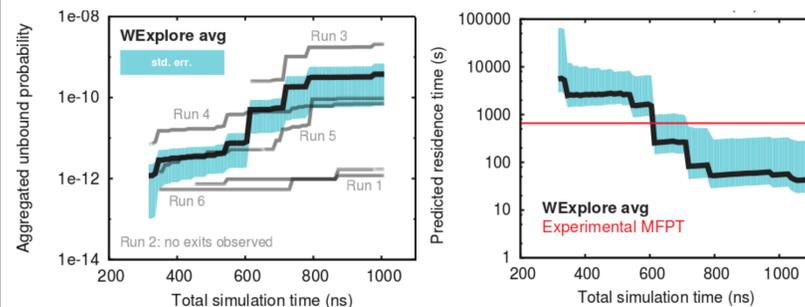## Solvent Accessible Surface Area

SASA 525.0 / 69.0

Solvent accessible surface area (SASA) reports the progress of ligand unbinding. The free energy of the network shows that the upper branch (P_1) is more probable than the lower. P_1 is the shortest path leaving the wide sEH binding site.

Free Energy

Free Energy 34.0 / 0.0

Transition State Ensemble (TSE)

Highest Flux Path / **Path Bottleneck Ensemble**

Crystal Structure Pose (PDB:4OD0)

LHS — Center Pinch — RHS
Residue (Not Shown)
Ligand — Donor — Acceptor

Unbound Committor Probability

p_u 1.0 / 0.0

Unbound Sink
Transition State Ensemble
Bound Source

Committor probabilities are calculated using the source and sink nodes shown above (5). The transition state is defined as the nodes with a committor value between 0.4 and 0.6.

Residence Time

WExplore avg / std. err. / Run 3 / Run 4 / Run 5 / Run 6 / Run 1 / Run 2: no exits observed

Aggregated unbound probability vs Total simulation time (ns)

WExplore avg / Experimental MFPT
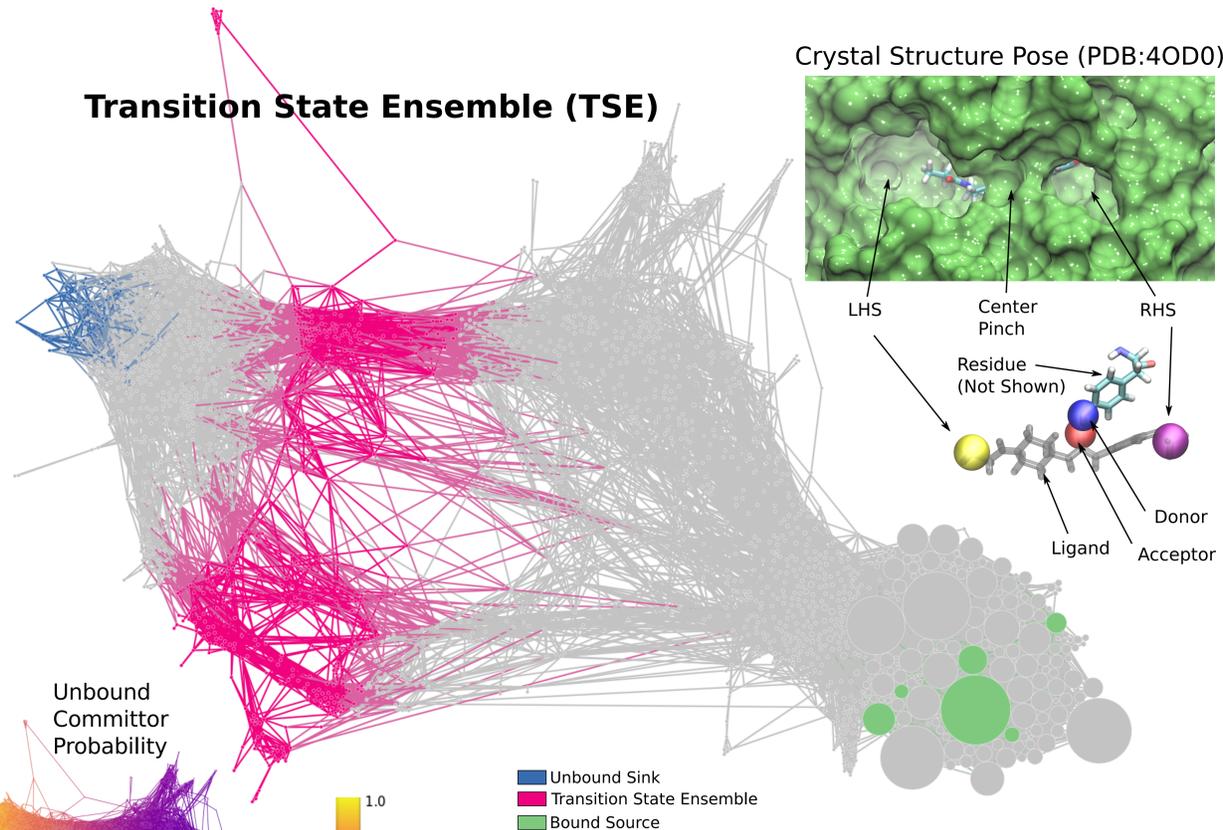
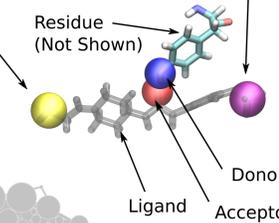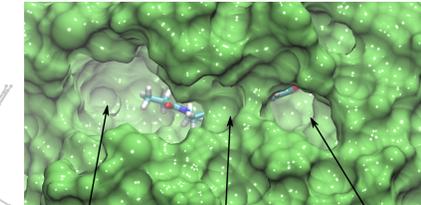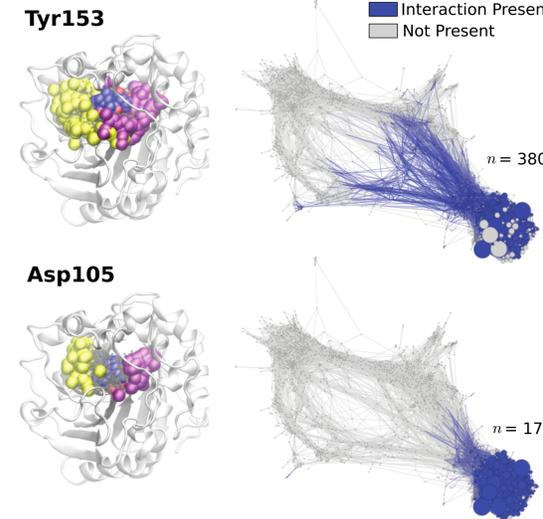Predicted residence time (s) vs Total simulation time (ns)

The sampling method used was WExplore (8) a variation on the weighted ensemble method of Huber and Kim (9). Six independent simulations were run accumulating a total of 7.0 microseconds of sampling in which 75 exit points were observed. Residence times (1/MFPT) were calculated from the time for each exit point. Our final value (42 s) is relatively close to the experimental value (11 min) given the timescales involved.

Network Communities

R_2 / R_1 / U / P_1^* / P_1 / B^* / B_1 / P_2 / B_2 / *

Using a network modularity metric the network was divided up into distinct communities. The main communities are B_1 (bound), B^* (exit branchpoint), P_1 (path 1), P_2 (path 2), and U (unbound). R_1 and R_2 were found to have a reversal in ligand orientation. Profiling hydrogen bonds of each community shows that **in the bound state a handful of interactions are present in high frequency, while in B^* fewer interactions are stabilizing (Tyr153).** In P_1 interactions are more diverse but occur less frequently and are distinct from all other communities. The unbound ensemble has a large number of low frequency interactions as would be expected. Gray bars indicate interactions highlighted in the boxes to the right.

Hydrogen Bond Frequency Patterns

H-Bond Acceptor Donor Pairs Sorted by Frequency

B_1 / B_2 / B^* / n / P_1 / P_1^* / P_2 / U

Bound State Stabilizing Interactions

Interaction Present / Not Present
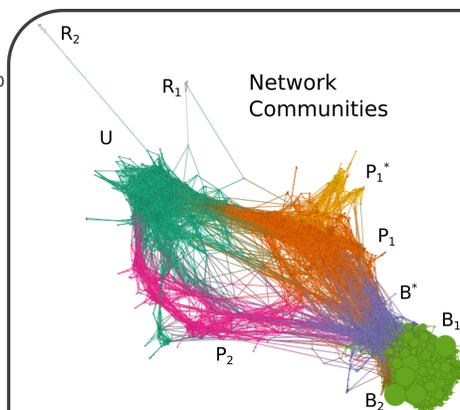
Tyr153 — $n = 380$

Asp105 — $n = 172$

Hydrogen bonds in the native state play different roles. Asp105 only stabilizes the fully bound state, while Tyr153 stabilizes a larger set of states, facilitating outward movement of the ligand.
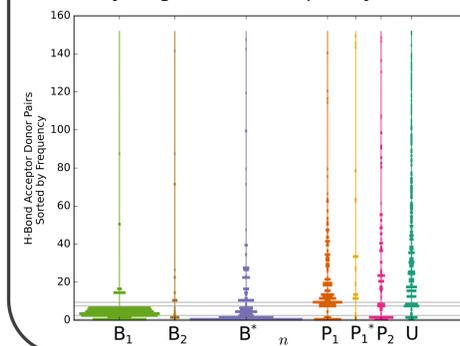
Transition State Stabilizing Interactions

Val268 — $n = 71$

Met189 — $n = 51$
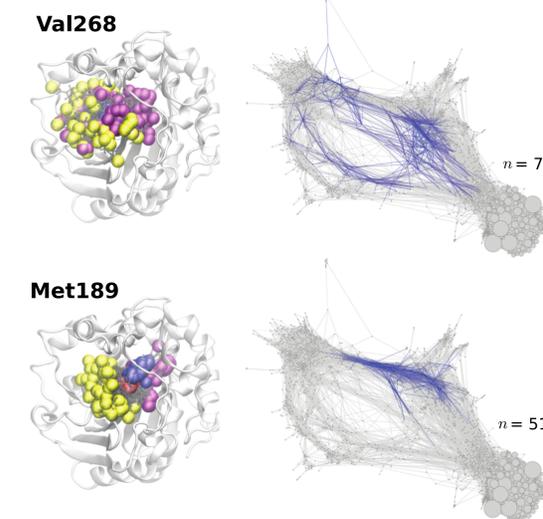
Interactions with specific ligands stabilize different ligand exit pathways at the overall transition state. Met189 stabilizes movement along the RHS of the binding site (P_1) and is the highest frequency interaction in P_1 coincident with the TSE. Val268 stabilizes ligand poses at the center of the binding site straddling both P_1 and P_2. **Met189 is likely a stabilizer of bottlenecks that determine (un)binding kinetics for inhibitors of sEH.**

Literature Cited

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