Using Free Energy Perturbation Calculations to Model the Mutation of

LeuT\textsubscript{Aa} and mDAT Residues that Bind TCAs

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Dopamine transporter (DAT) is a membrane-bound active transporter that primarily functions to uptake dopamine from the interneural synapse into the presynaptic terminal by cotransporting Na\textsuperscript{+} and Cl\textsuperscript{−} ions down their concentration gradients. Dopaminergic pathways comprise a significant portion of the motor control and reward systems of the brain, and aberrant DAT has been implicated in several diseases of these systems, including Parkinsonism\textsuperscript{1}. The transporter is the target of several drugs, including attention deficit-hyperactivity disorder (ADHD) medications\textsuperscript{2}, cocaine (which acts as a competitive dopamine inhibitor)\textsuperscript{3}, and amphetamines (which trigger dopamine efflux through the transporter)\textsuperscript{4}.

DAT is categorized within the neurotransmitter sodium symporter (NSS) family, the members of which all utilize electrochemical gradients to concentrate within a cell several substrates, including: the monoamines dopamine, norepinephrine, and serotonin; the amino acids GABA, glycine, proline, leucine, and taurine; and osmolites betaine and creatine\textsuperscript{5}. Yamashita et al. were the first to determine the structure of a bacterial homologue to the NSS proteins—the leucine transporter of \textit{Aquifex aeolicus}\textsuperscript{6}. Prior to this characterization, several molecular models of DAT were based on the structures of proteins from other families\textsuperscript{7,8}; the LeuT\textsubscript{Aa} structure has allowed for more extensive modeling studies of DAT\textsuperscript{9,10,11}. 

Crystal structures of LeuT<sub>Aa</sub> bound to the tricyclic antidepressants (TCAs) desipramine<sup>12</sup>, clomipramine<sup>13</sup>, and imipramine<sup>13</sup> have been recently reported. The structures of these ligands are similar (Figure 1), and they were crystallized bound to the same, non-competitive site (Table 1). Further study of this binding site in LeuT<sub>Aa</sub> may lead to elucidation of the inhibition mechanism of one or more of the aforementioned hDAT inhibitors, as well as support further drug design efforts focused on modulation of hDAT function.

To better understand the LeuT<sub>Aa</sub>–TCA complex, we will perform free energy perturbation (FEP) calculations, which estimate the relative binding energy of each residue in the inhibitor binding site. Similar calculations will then be run on a mouse DAT (mDAT) model based on the LeuT<sub>Aa</sub>–TCA structures<sup>14</sup>. These calculations will be compared to experimental data obtained from mutagenesis studies performed on mDAT, and the results will be used to validate or refine the mDAT model.

**Computational Methods**

The FEP calculations will be performed with NAMD 2.7b1<sup>15</sup>. The calculation is comprised of a series of molecular dynamics (MD) simulations, separated into units called windows. Each window will contain the protein with the target residue in an intermediate form between the original and final residue. For example, during modeling of the of the LeuT<sub>Aa</sub> F253A mutation, one window would represent the 253<sup>rd</sup> residue as 25% phenylalanine and 75% alanine. Whereas this is physically unrealistic, it allows for the energies of the change from wild-type protein to mutant to be...
progressively modeled. Each MD simulation window will be run for 20 picoseconds using a parameterized CHARMM/OPLS force field, and the free energy of each window will be determined as

\[
\Delta G = -k_B T \ln \left\langle \exp \left( \frac{U - U_0}{k_B T} \right) \right\rangle
\]

where \( U \) is energy, \( k_B \) is Boltzmann’s constant, and \( T \) is temperature in Kelvin. The Mutator 1.0 plugin in NAMD will be used to create the necessary hybrid residue input files.

The structures used for the LeuT\(_{Aa}\)–TCA calculations will be obtained from the RCSB Protein Data Bank and rebuilt as necessary to match the wild-type primary structure. For the mDAT calculations, a homology model\(^1\) will be used. All simulations will be performed in a water box with periodic boundary conditions and a 2 femtosecond timestep, using the rigidBonds option to freeze hydrogen-heavy atom vibrations. Though these are membrane-bound proteins, the simulations will be run without a model membrane in order to decrease computational cost. This should not adversely affect the calculated results because the system is not expected to move significantly within the window simulation duration.

The LeuT\(_{Aa}\) residues identified as important for TCA binding are listed in Table 1 along with their mDAT homologues. The LeuT\(_{Aa}\) system will be examined first, with results compared to mutagenesis literature values, to acclimate the experimenter with MD simulations and FEP calculations. There are 90 potential FEP calculations suggested by the Table 1 (the mutation of the 15 LeuT\(_{Aa}\) and corresponding 15 mDAT residues while bound to each of the three TCAs), and selection among these will be guided by the availability of corresponding experimental results and computational resources.
**Expected Results**

Because the calculations that will be performed are designed to estimate the free energy change due to a residue mutation, a direct comparison with mutagenesis studies can be made. In comparing the LeuT<sub>Aa</sub> FEP predictions with experiment, a strong correlation is expected, because the LeuT<sub>Aa</sub>–TCA models will be built from XRD data. These results should be quantitative, but only a qualitative one may be observed for various reasons. If the correlation between the mDAT homologue model and mutagenesis studies is as strong as those obtained with LeuT<sub>Aa</sub>, it would support the proposed mDAT model. If discrepancies are found between the model predictions and experiment, they may serve to refine the mDAT computational model.

<table>
<thead>
<tr>
<th>LeuT&lt;sub&gt;Aa&lt;/sub&gt; Residue</th>
<th>Type of Binding in Crystal Structure</th>
<th>FEP Calculation Target Residue</th>
<th>mDAT homologue Residues**</th>
</tr>
</thead>
<tbody>
<tr>
<td>L25</td>
<td>---*</td>
<td>Hydrophobic w/Ring 3</td>
<td>Ala L80</td>
</tr>
<tr>
<td>L29</td>
<td>---</td>
<td>Hydrophobic w/Ring 1</td>
<td>Ala W84</td>
</tr>
<tr>
<td>R30</td>
<td>Cation-π w/Ring 3 and F253; Salt Bridge w/D404</td>
<td>Cation-π w/Ring 3 and F253; Salt Bridge w/D404</td>
<td>Ala R85</td>
</tr>
<tr>
<td>V33</td>
<td>---</td>
<td>Hydrophobic w/Ring 1</td>
<td>Ala Y88</td>
</tr>
<tr>
<td>Q34</td>
<td>Hydrophobic w/Ring 3</td>
<td>Polar w/Cl</td>
<td>Ala L89</td>
</tr>
<tr>
<td>V104</td>
<td>---</td>
<td>Hydrophobic w/Ring 3</td>
<td>Ala V152</td>
</tr>
<tr>
<td>Y107</td>
<td>---</td>
<td>Hydrophobic w/Ring 3</td>
<td>Ala F155</td>
</tr>
<tr>
<td>Y108</td>
<td>---</td>
<td>Hydrophobic w/Ring 3</td>
<td>Ala Y156</td>
</tr>
<tr>
<td>I111</td>
<td>---</td>
<td>Hydrophobic w/Tail</td>
<td>Ala I159</td>
</tr>
<tr>
<td>F253</td>
<td>Hydrophobic w/Ring 1; cation-π w/R30</td>
<td>Hydrophobic w/Ring 3</td>
<td>Ala F319</td>
</tr>
<tr>
<td>A319</td>
<td>Hydrophobic w/Rings 1 and 2</td>
<td>Displaced “upward”</td>
<td>Gly G385</td>
</tr>
<tr>
<td>F320</td>
<td>Hydrophobic w/Ring 2 (azepine) and tail</td>
<td>Hydrophobic w/Tail</td>
<td>Ala P386</td>
</tr>
<tr>
<td>L400</td>
<td>Hydrophobic w/tail</td>
<td>Hydrophobic w/tail</td>
<td>Ala F471</td>
</tr>
<tr>
<td>D401</td>
<td>Electrostatic w/tail (potential salt bridge)</td>
<td>Ionic w/tail</td>
<td>Ala T472</td>
</tr>
<tr>
<td>D404</td>
<td>Salt Bridge w/R30</td>
<td>Salt Bridge w/R30</td>
<td>Ala D475</td>
</tr>
</tbody>
</table>

*"---" indicates that the specific interaction was not commented upon in the citation
** Determined via a BLASTP 2.2.21+ alignment
References


