Using Free Energy Perturbation Calculations to Model the Mutation of $LeuT_{Aa}$ and mDAT Residues that Bind TCAs

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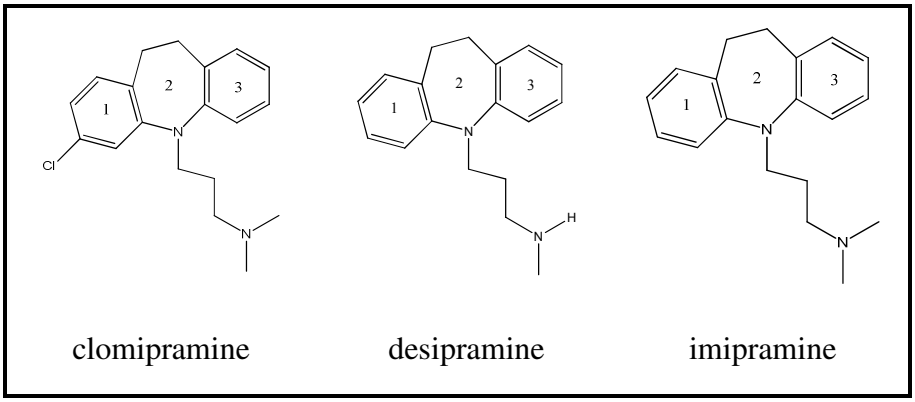
Dopamine transporter (DAT) is a membrane-bound active transporter that primarily functions to uptake dopamine from the interneural synapse into the presynaptic terminal by cotransporting Na^+ and Cl^- ions down their concentration gradients. Dopaminergic pathways comprise a significant portion of the motor control and reward systems of the brain, and aberrant DAT has been implicated in several diseases of these systems, including Parkinsonism[1]. The transporter is the target of several drugs, including attention deficit-hyperactivity disorder (ADHD) medications[2], cocaine (which acts as a competitive dopamine inhibitor)[3], and amphetamines (which trigger dopamine efflux through the transporter)[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[5]. Yamashita et al. were the first to determine the structure of a bacterial homologue to the NSS proteins—the leucine transporter of *Aquifex aeolicus*[6]. Prior to this characterization, several molecular models of DAT were based on the structures of proteins from other families[7,8]; the $LeuT_{Aa}$ structure has allowed for more extensive modeling studies of DAT[9,10,11].

Crystal structures of $LeuT_{Aa}$ bound to the tricyclic antidepressants (TCAs) desipramine[12], clomipramine[13], and imipramine[13] 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 $LeuT_{Aa}$ 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 $LeuT_{Aa}$–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 $LeuT_{Aa}$-TCA structures[14]. 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.

Figure 1. Structures of hDAT Inhibitors: Tricyclic Antidepressants



❖ Computational Methods

The FEP calculations will be performed with NAMD 2.7b1[15]. 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 $LeuT_{Aa}$ F253A mutation, one window would represent the 253rd 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

$$\Delta G = -k_B T \ln \left\langle \exp\left\{ \frac{U_1 - U_0}{k_B T} \right\} \right\rangle$$

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[14] 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 LeuTAa 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 $LeuT_{Aa}$ FEP predictions with experiment, a strong correlation is expected, because the $LeuT_{Aa}$–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 $LeuT_{Aa}$, 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 1. Important Residues for LeuTAa – TCA Binding and their Free Energy Perturbation Calculation Target Mutations and mDAT Homologue Residues

$LeuT_{Aa}$ Residue	Type of Binding in Crystal Structure		FEP Calculation Target Residue	mDAT homologue Residues**
	desipramine[12]	clomipramine and imipramine[13]		
L25	---*	Hydrophobic w/Ring 3	Ala	L80
L29	---	Hydrophobic w/Ring 1	Ala	W84
R30	Cation-π w/Ring 3 and F253; Salt Bridge w/D404	Cation-π w/Ring 3 and F253; Salt Bridge w/D404	Ala	R85
V33	---	Hydrophobic w/Ring 1	Ala	Y88
Q34	Hydrophobic w/Ring 3	Polar w/Cl	Ala	L89
V104	---	Hydrophobic w/Ring 3	Ala	V152
Y107	---	Hydrophobic w/Ring 3	Ala	F155
Y108	---	Hydrophobic w/Ring 3	Ala	Y156
I111	---	Hydrophobic w/Tail	Ala	I159
F253	Hydrophobic w/Ring 1; cation-π w/R30	Hydrophobic w/Ring 3	Ala	F319
A319	Hydrophobic w/Rings 1 and 2	Displaced "upward"	Gly	G385
F320	Hydrophobic w/Ring 2 (azepine) and tail	Hydrophobic w/Tail	Ala	P386
L400	Hydrophobic w/tail	Hydrophobic w/tail	Ala	F471
D401	Electrostatic w/tail (potential salt bridge)	Ionic w/tail	Ala	T472
D404	Salt Bridge w/R30	Salt Bridge w/R30	Ala	D475

*"---" indicates that the specific interaction was not commented upon in the citation

** Determined via a BLASTP 2.2.21+ alignment[16]

❖ References

1. Uhl, R. G. **2003**. Dopamine transporters: basic science and human variation of a key molecule for dopaminergic function, locomotion, and Parkinsonism. *Movement Disorders*. 18: S71-S80.

2. Madras, B.K., Miller, G.M., Fischman, A.J. **2004**. The dopamine transporter and attention deficit/hyperactivity disorder. *Biological Psychiatry*. 57 (11): 1397-1409.

3. Earles, C. and Schenk, J. O. **1999**. Multisubstrate mechanism for the inward transport of dopamine by the human dopamine transporter expressed in HEK cells and its inhibition by cocaine. *Synapse*. 33 (3): 230-238.

4. Kahlig, K. M., Binda, F., Khoshbouei, H., Blakely, R. D., McMahon, D. G., Javitch, J. A., and Galli, A. **2005**. Amphetamine induces dopamine efflux through a dopamine transporter channel. *PNAS*. 102 (9): 3495-3500.

5. Masson, J., Sagné, C., Hamon, M., El mestikawy, S. **1999**. Neuotransmitter transporters in the central nervous system. *Pharmacological Reviews*. 51 (3): 439-464.

6. Yamashita, A., Singh, S.K., Kawate, T., Jin, Y., Gouaux, E. **2005**. Crystal structure of a bacterial homologue of Na^+/Cl^- -depenent neurotransmitter transporters. *Nature*. 437: 215-223.

7. Ravna, A. W., Sylte, I., and Dahl, S.G. **2003**. Molecular model of the neural dopamine transporter. *Journal of Computer Aided Molecular Design*. 17: 367-382.

8. Ravna, A. W., Sylte, I., Kristiansen, K., and Dahl S.G. **2006**. Putative drug binding conformations of monoamine transporters. *Bioorganic & Medicinal Chemistry*. 14:666-675.

9. Huang, X., Zhan, C. **2007**. How dopamine transporter interacts with dopamine: Insights from molecular modeling and simulation. *Biophysical Journal*. 93: 3627-3639.

10. Kniazeff, J., Shi, L., Loland, C.J., Javitch, J.A., Weinstein, H., Gether, U. **2008**. An intracellular interaction network regulates conformational transitions in the dopamine transporter. *Journal of Biological Chemistry*. 283 (25): 17691-17701.

11. Beuming, T., Kniazeff, J., Bergmann, M.L., Shi, L., Gracia, L., Raniszewska, K., Newman, A.H., Javitch, J.A., Weinstein, H., Gether, U., and Loland, C.J. **2008**. The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nature Neruoscience*. 11: 780-789.

12. Zhou, Z., Zhen, J., Karpowich, N.K., Goetz, R.M., Law, C.J., Reith, M.E.A., and Wang, D. **2007**. LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. *Science*. 317: 1390-1393.

13. Singh, S.K., Yamashita, A., and Gouaux, E. **2007**. Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. *Nature*. 448: 952-956.

14. Indarte, M., Madura, J.D., and Surratt, C.K. **2008**. Dopamine transporter comparative molecular modeling and binding site prediction using the $LeuT_{Aa}$ leucine transporter as a template. *Proteins: Structure, Function, and Bioinformatics*. 70: 1033-46.

15. Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kale, L., and Schulten, K. **2005**. Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry*. 26: 1781-1802.

16. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. **1997**. Gapped BLAST and PSI-BLASST: A new generation of protein database search programs. *Nucleic Acids Research.* 25: 3389-3402.