

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

Chad Miller
Youngstown State University

Dr. Jeffry D. Madura
Center for Computational Sciences
Department of Chemistry & Biochemistry
Duquesne University

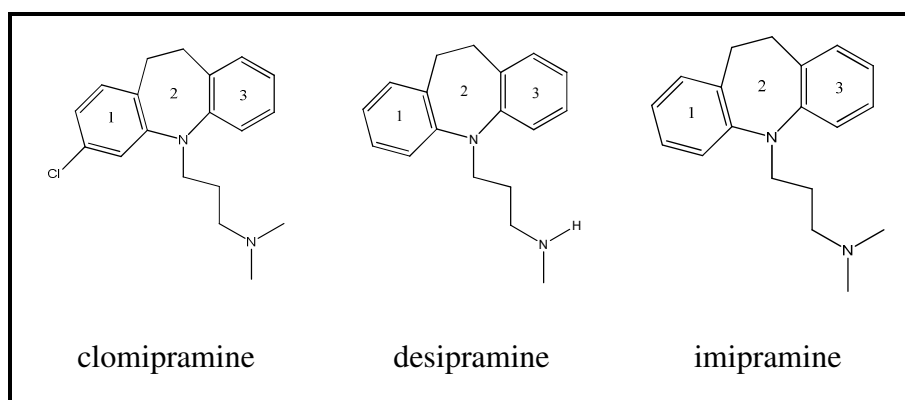
Dopamine transporter (DAT) are membrane-bound active transporters that primarily function to uptake dopamine from the interneural synapse into the presynaptic terminal by cotransporting Na⁺ and Cl⁻ ions down their concentration gradients. Dopaminergic pathways comprise a significant portion of the motor control and reward systems of the brain, and aberrant DAT has been implicated in several diseases of these systems, including Parkinsonism¹. The transporter is the target of several drugs, including attention deficit-hyperactivity disorder (ADHD) medications², 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 creatine⁵. 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) desipramine¹², clomipramine¹³, and imipramine¹³ 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

Figure 1. Structures of hDAT Inhibitors: Tricyclic Antidepressants



calculations will then be run on a mouse DAT (mDAT) model based on the LeuT_{Aa}-TCA structures¹⁴. 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 2.7b1¹⁵. 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 potential 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 model¹⁴ will be used. All simulations will be performed in a water box with periodic boundary conditions and a 2 femtosecond timestep, using rigidBonds 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 should not 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 48 potential FEB calculations suggested by the Table 1 (the mutation of the 16 LeuT_{Aa} and 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+ alignment¹⁶

❖ 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**. Neurotransmitter 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⁻-dependent 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 Neuroscience*. 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-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*. 25: 3389-3402.