HOW DOPAMINE TRANSPORTER INTERACTS WITH DOPAMINE: INSIGHTS FROM MOLECULAR MODELING AND SIMULATION

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Dopamine Transporter Physiology

- Transport dopamine from the synaptic cleft into the pre-synaptic terminal
- Requires 2 Na\(^+\) and 1 Cl\(^-\) per to move a molecule of dopamine against its electrochemical gradient

http://anthropologynet.files.wordpress.com/2008/01/neuron-synapse.png?w=350
DAT as a Therapeutic Target

- Parkinson’s Disease, a condition of decreased dopaminergic action
- The endogenous cocaine receptor
  - Cocaine inhibits hDAT, allowing dopamine to remain in the synapses longer
  - Because dopamine is associated with reward pathways, cocaine is very reinforcing
DAT Structural Homology

- A member of the neurotransmitter sodium symporters (NSS) family
  - Transport neurotransmitters into and out of the neural synapse, usually using sodium and chloride electrochemical gradients
- DAT has been computationally modeled, but with a structure based on various NSS family members, but models based on the recent bacterial leucine transporter should give better results.
Building the DAT Model

- LeuT and DAT primary sequences aligned based on the Blosum scoring function
  - Sequence identity: 20.4%
  - Sequence homology: 42.0%
    - Based on structurally conserved residues within the NSS family
- 3D model was then built with the InsightII
The Lipid Bilayer

- POPC layer from the VMD software
- Gaussian 03
  - HF/6-31G*
  - Geometry minimization
  - Restrained electrostatic potential (RESP) calculations

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)
Completing the Computational Box

- TIP3P water with 4 Na\(^+\) atoms; minimum solute wall distance 10 Å
- DAT residues in physiological state (i.e., at pH ~ 7.4)
- Box dimensions approx. 126 Å X 125 Å 118 Å; 154,114 atoms
- Several steps of energy minimization and short molecular dynamics calculations

http://www.ucalgary.ca/~tieleman/images/sideview.jpg
Binding Dopamine to DAT

- DOCK 5.4: matches various confirmers of DAT to the binding area
- AutoDock 3.0.5: uses a monte carlo approach with binding energy calculations
- To allow for protein flexibility, MD simulations were run, calculating electrostatic and van der Waals energies, narrowing the dopamine geometries to two candidates
Calculation of Binding Free Energy

- MM-PBSA Method
  - MM: molecular mechanics energy calculation
  - PB: Poisson-Boltzmann method for determining electrostatic energies in ionic solutions
  - SA: calculates the non-polar solvation free energy based on the solvent accessible surface area
- Lowest binding free energy DAT-dopamine complex was used for molecular dynamics

\[
\Delta G_{\text{bind}} = \Delta E_{\text{bind}} - T\Delta S
\]
\[
\Delta E_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}}
\]
\[
\Delta G_{\text{solv}} = \Delta G_{\text{PB}} + \Delta G_{\text{np}}
\]
\[
\Delta G_{\text{np}} = \gamma S \text{ASA}
\]
Molecular Dynamics

- Performed on AMBER8
  - Heated to 300 K by the weak coupling method and equilibrated for approx. 49 ps
  - The particle-mesh Ewald method was used to treat electrostatic interactions at long range
  - Time step 2 fs (SHAKE algorithm used for atoms bonded to hydrogen)
DAT Structure

- Helices 1-10 likely transporting core
- Helix 12 may form DAT dimers/tetramers
- This structure model differs from previous
  - A different homologue was used (i.e., LeuT)
  - 2 Na⁺ ions placed in the structure
  - Lipid bilayer and solvent accounted for during energy minimization
Arg-Asp Salt Bridge

- Based on LeuT crystal structure, an Arg$^{85}$ – Asp$^{476}$ salt bridge was suggested as an obstacle for the substrate.
- This bridge was not generally present in the MD simulation.
Coordination of Na\(^+\) Ions

- Na\(^+\) ions may serve to stabilize the protein core and two unwound helices
- May also assist in large-scale conformational changes
- Coordination changes when dopamine is bound
# Coordination of Na⁺ Ions

## TABLE 1  Coordination details for the Na⁺ ions during the MD simulations for both the DAT model and its complex structure with dopamine

<table>
<thead>
<tr>
<th>Ions</th>
<th>Residue</th>
<th>Atom</th>
<th>Fraction</th>
<th>Residue</th>
<th>Atom</th>
<th>Fraction</th>
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</thead>
<tbody>
<tr>
<td>Na₁</td>
<td>Phe⁷⁶</td>
<td>O=C</td>
<td>0.735</td>
<td>Ala⁷⁷</td>
<td>O=C</td>
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<td>Ala⁷⁷</td>
<td>O=C</td>
<td>0.988</td>
<td>Asp⁷⁹</td>
<td>OD1</td>
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<tr>
<td></td>
<td>Asn⁸²</td>
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<td>Asp⁸³</td>
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<tr>
<td></td>
<td>Ser³²¹</td>
<td>O=C</td>
<td>0.991</td>
<td>Phe³²⁰</td>
<td>O=C</td>
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<tr>
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<td>Asp³⁵³</td>
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<td>Ser³²¹</td>
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<tr>
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<tr>
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<td></td>
<td>6: 0.580</td>
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<td>5: 0.963</td>
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<td></td>
<td></td>
<td>6: 0.030</td>
<td></td>
<td></td>
<td>6: 0.030</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ions</th>
<th>Residue</th>
<th>Atom</th>
<th>Fraction</th>
<th>Residue</th>
<th>Atom</th>
<th>Fraction</th>
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</thead>
<tbody>
<tr>
<td>Na₂</td>
<td>Gly⁷⁵</td>
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<td>0.965</td>
<td>Gly⁷⁵</td>
<td>O=C</td>
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<tr>
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<td>Asp⁷⁹</td>
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<td>Leu⁴¹⁸</td>
<td>O=C</td>
<td>0.911</td>
<td>Leu⁴¹⁸</td>
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<td>Asp³²¹</td>
<td>OD1</td>
<td>1.000</td>
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<tr>
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<td>Asp³⁵¹</td>
<td>OD2</td>
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<td>Asp³⁵¹</td>
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<tr>
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<td>6: 0.780</td>
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<td>5: 0.949</td>
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</tbody>
</table>

A distance cutoff of 3 Å was used for the coordination criterion, and the fraction was calculated as the ratio of the number of snapshots with the coordination to the total number of snapshots taken from the stable MD trajectory. The fraction of each coordination number (4, 5, or 6) was calculated similarly.
The DAT-dopamine Complex

- Dopamine was located in a dehydrated pocket
- In the DAT-dopamine complex, the aforementioned Arg$^{85}$ – Asp$^{476}$ salt bridge was largely present
- The hexacoordination of Na$^{+}$ becomes pentacoordination
DAT-dopamine Interactions

- Asp\textsuperscript{79} moves from coordination of Na\textsuperscript{+} to a hydrogen bond with the cationic end of dopamine
- Hydrogen bonding, hydrophobic contacts, pi-stacking, and cation-pi interaction
Comparison of Model to Mutagenesis Studies

- Asp$^{79}$\rightarrow{Ala or Gly} abolish dopamine reuptake, Asp$^{79}$\rightarrow{Glu} significantly reduces activity.
- Asp$^{313}$\rightarrow{Asn} increases $K_M$. Located near the cation head, a change from -1 to 0 weakens binding.
- Lys$^{257}$\rightarrow{Ala} and Arg$^{283}$\rightarrow{Ala} decrease $K_M$. These positive residues near the cationic head become neutral, destroying the repulsion and increasing binding.
- Phe$^{155}$\rightarrow{Ala}, Trp$^{84}$\rightarrow{Leu}, Leu$^{104}$\rightarrow{Val}, Phe$^{105}$\rightarrow{Cys}, and Ala$^{109}$\rightarrow{Val} have been reported to have no measurable effect on $K_M$; this is likely because these residues are far from the binding site.
Comparison of $\Delta G_{\text{bind}}$ with Experiment

<table>
<thead>
<tr>
<th></th>
<th>Initial DAT complex</th>
<th>DAT complex during MM/MD calculations</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta G_{\text{bind}}$ (kcal/mol)</td>
<td>-5.6</td>
<td>-6.4</td>
<td>-7.4</td>
</tr>
<tr>
<td>$K_d$ ($\mu$m)</td>
<td>78</td>
<td>20</td>
<td>---</td>
</tr>
<tr>
<td>$K_M$ ($\mu$m)</td>
<td>---</td>
<td>---</td>
<td>3.466 ± 0.200</td>
</tr>
</tbody>
</table>

Error may be due to explicitly defining solvent molecules during MM/MD calculations, but using a continuum solvent model when calculating free binding energies.
Entry of Substrates

- DAT largely open to extracellular space
- Pocket covered by aromatic side chains
- A one hydrogen bond and the R85 and D476 side chains are situated above the pocket
- As Na⁺ enters, dopamine slides further down and the R-85/D476 salt bridge forms, stabilizing the complex
Potential Future Work

- Longer MD calculations could be run to allow for further relaxation of the system (duration in the present work ~2.4 ns)
- Beginning simulations from alternate starting points could demonstrate convergence on presented pose/binding confirmation
- Use of an alternative force field could further confirm reported mechanism of substrate binding