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

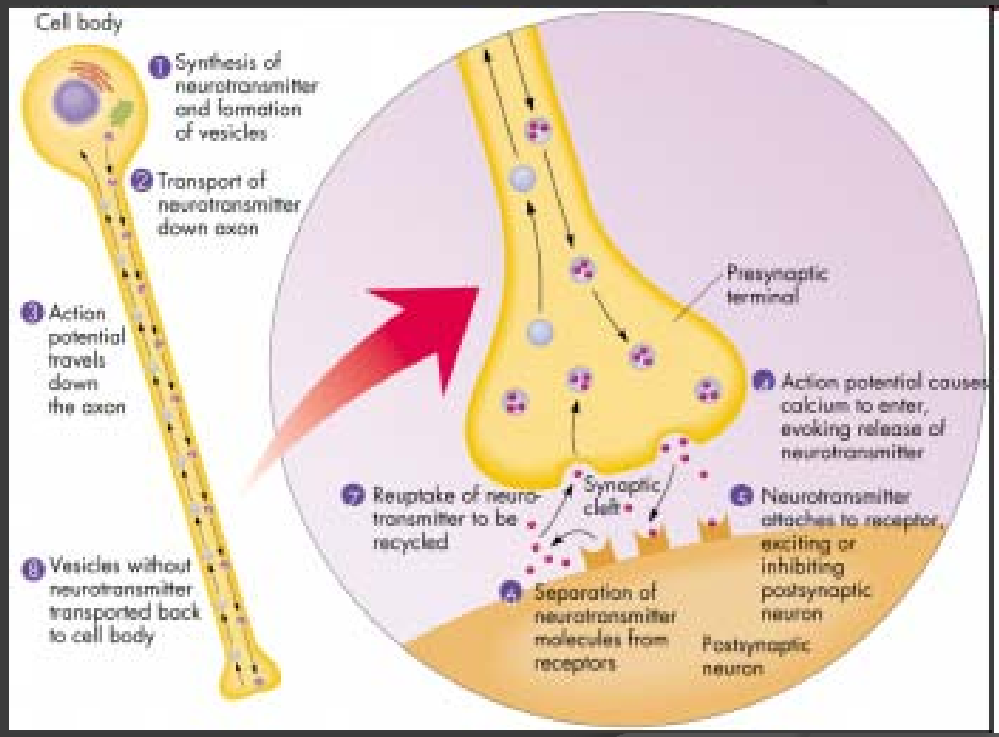
Authors: Xiaoqin Huang and Chang-Guo Zhan

Presented By: Chad Miller

Advisor: Dr. Jeffery Madura

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



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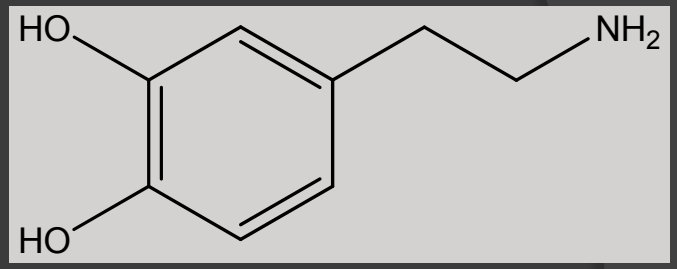
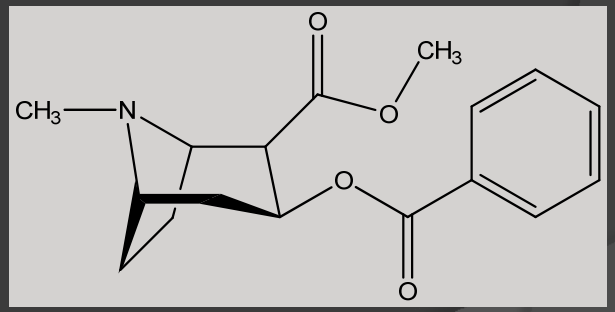
DAT as a Therapeutic Target

- 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



dopamine



cocaine

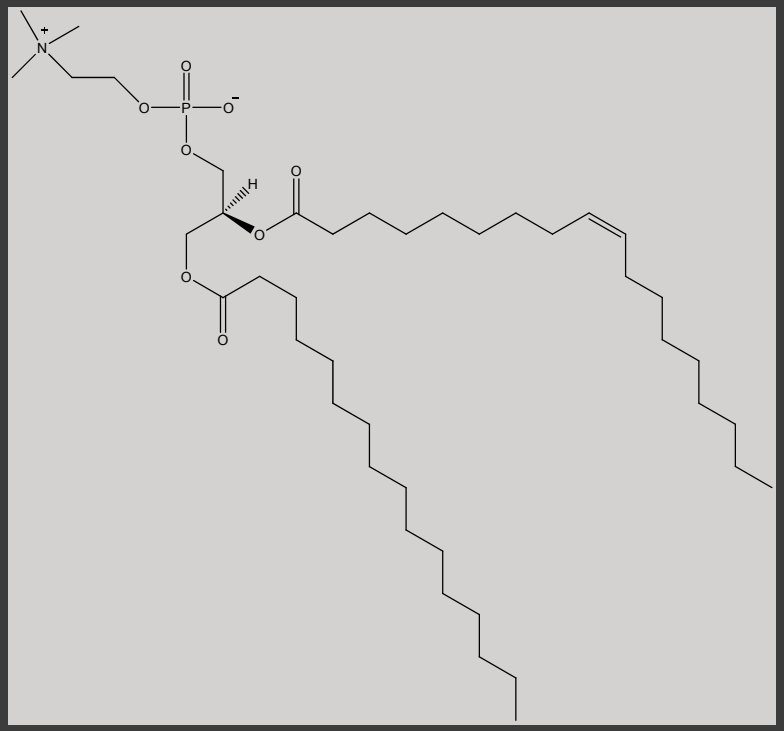
DAT Structural Homology

- 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

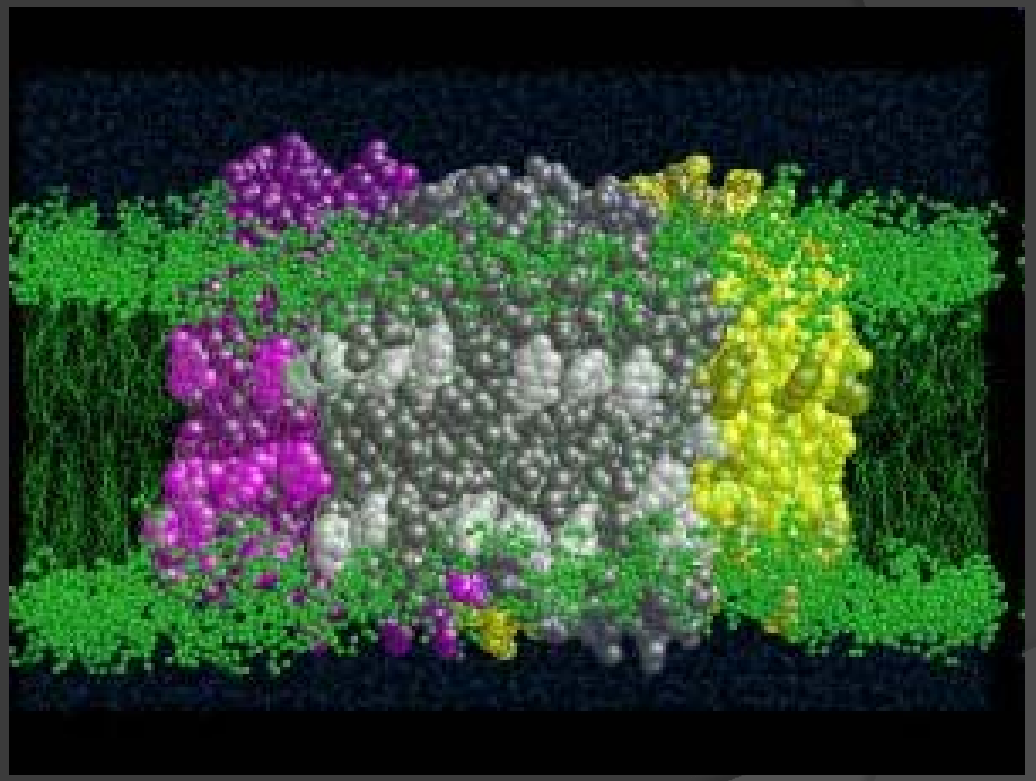


1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)

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

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

$$\Delta G_{bind} = \Delta E_{bind} - T\Delta S$$

$$\Delta E_{bind} = \Delta E_{MM} + \Delta G_{solv}$$

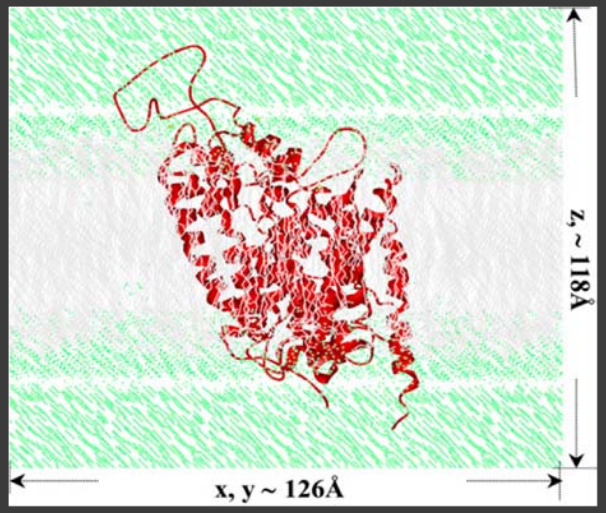
$$\Delta G_{solv} = \Delta G_{PB} + \Delta G_{np}$$

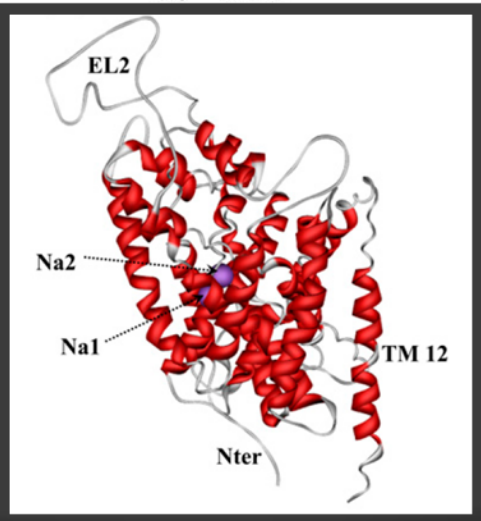
$$\Delta G_{np} = \gamma SASA$$

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

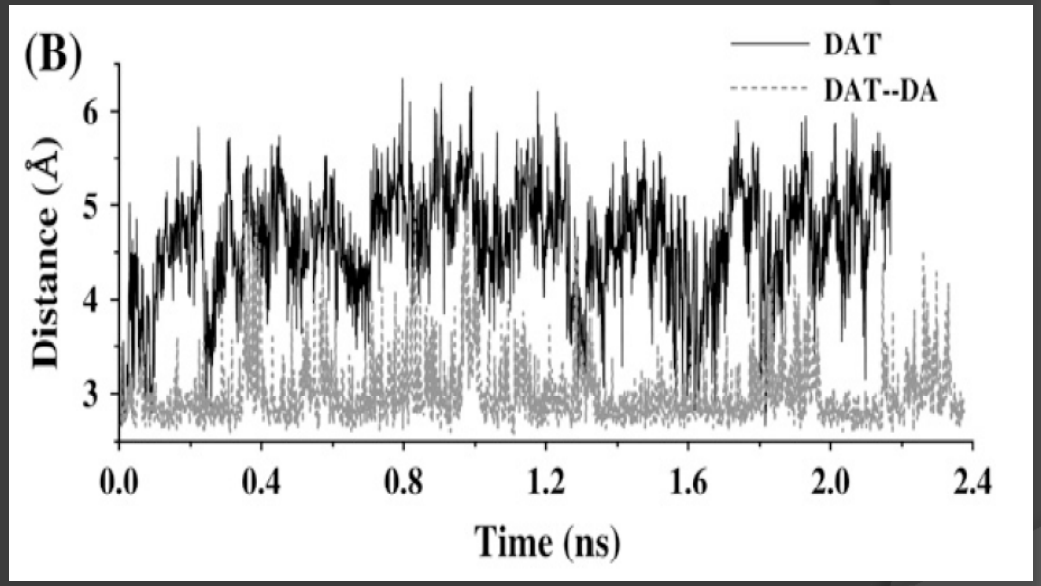




- Helices 1-10 likely transporting core
- Helix 12 may form DAT dimers/tetramers
- This structure model differs from previous
  - A different homologue was used (i.e., LeuT)
  - 2 Na^+ ions placed in the structure
  - Lipid bilayer and solvent accounted for during energy minimization

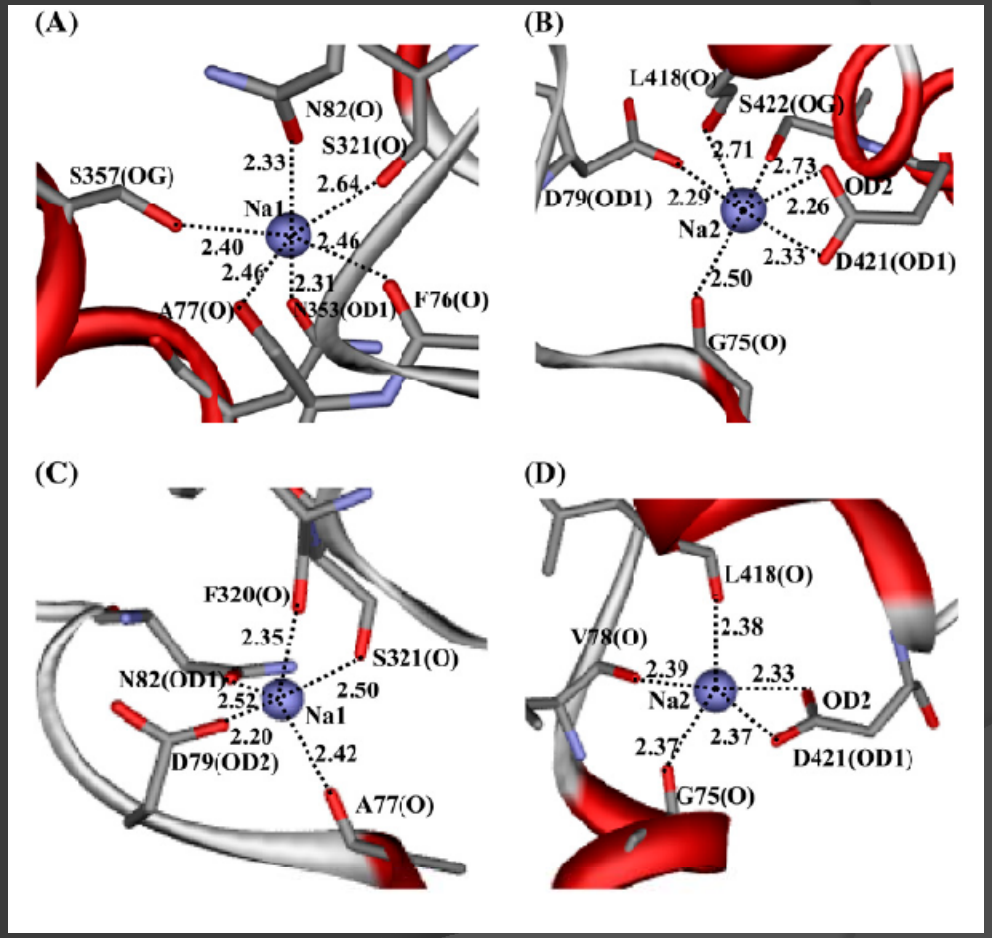
Arg-Asp Salt Bridge

- 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

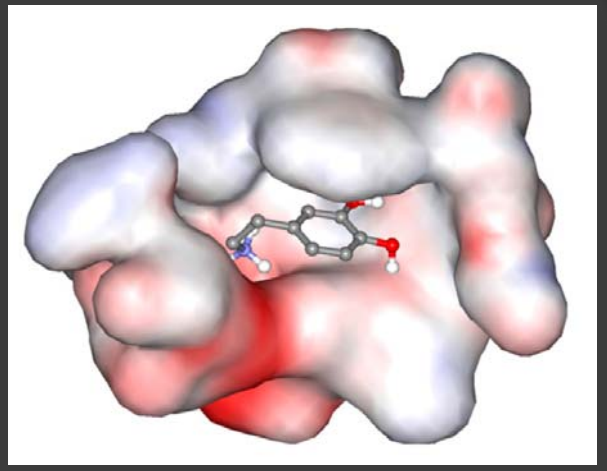
TABLE 1 Coordination details for the Na^+ ions during the MD simulations for both the DAT model and its complex structure with dopamine

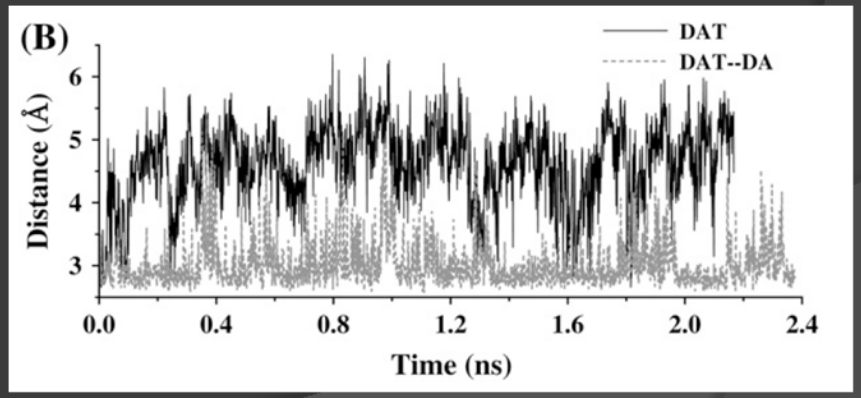
	DAT			DAT-dopamine complex		
Ions	Residue	Atom	Fraction	Residue	Atom	Fraction
Na1	Phe^{76}	O=C	0.735	Ala^{77}	O=C	1.000
	Ala^{77}	O=C	0.988	Asp^{79}	OD2	1.000
	Asn^{82}	OD1	0.998	Asn^{82}	OD1	1.000
	Ser^{321}	O=C	0.991	Phe^{320}	O=C	0.995
	Asn^{353}	O=C	1.000	Ser^{321}	O=C	0.944
	Ser^{357}	OG	0.965			
	Ser^{321}	OG	0.091			
	Coordination No. and its fraction		5: 0.397 6: 0.580	Coordination No. and its fraction		4: 0.006 5: 0.963 6: 0.030
Na2	Gly^{75}	O=C	0.965	Gly^{75}	O=C	1.000
	Asp^{79}	OD1	0.948	Val^{78}	O=C	1.000
	Leu^{418}	O=C	0.911	Leu^{418}	O=C	0.994
	Asp^{421}	OD1	1.000	Asp^{421}	OD1	1.000
	Asp^{421}	OD2	0.963	Asp^{421}	OD2	1.000
	Ser^{422}	OG	0.741			
	Val^{78}	O=C	0.032			
	Coordination No. and its fraction		5: 0.200 6: 0.780	Coordination No. and its fraction		4: 0.045 5: 0.949

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.

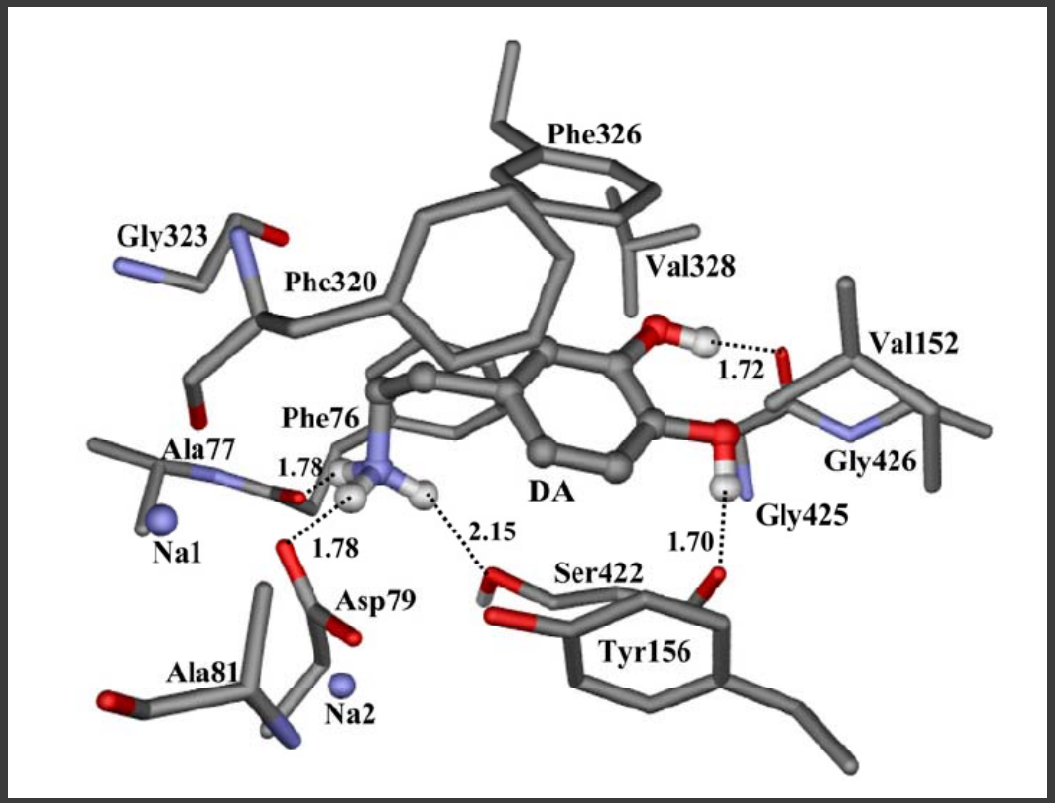
The DAT-dopamine Complex

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

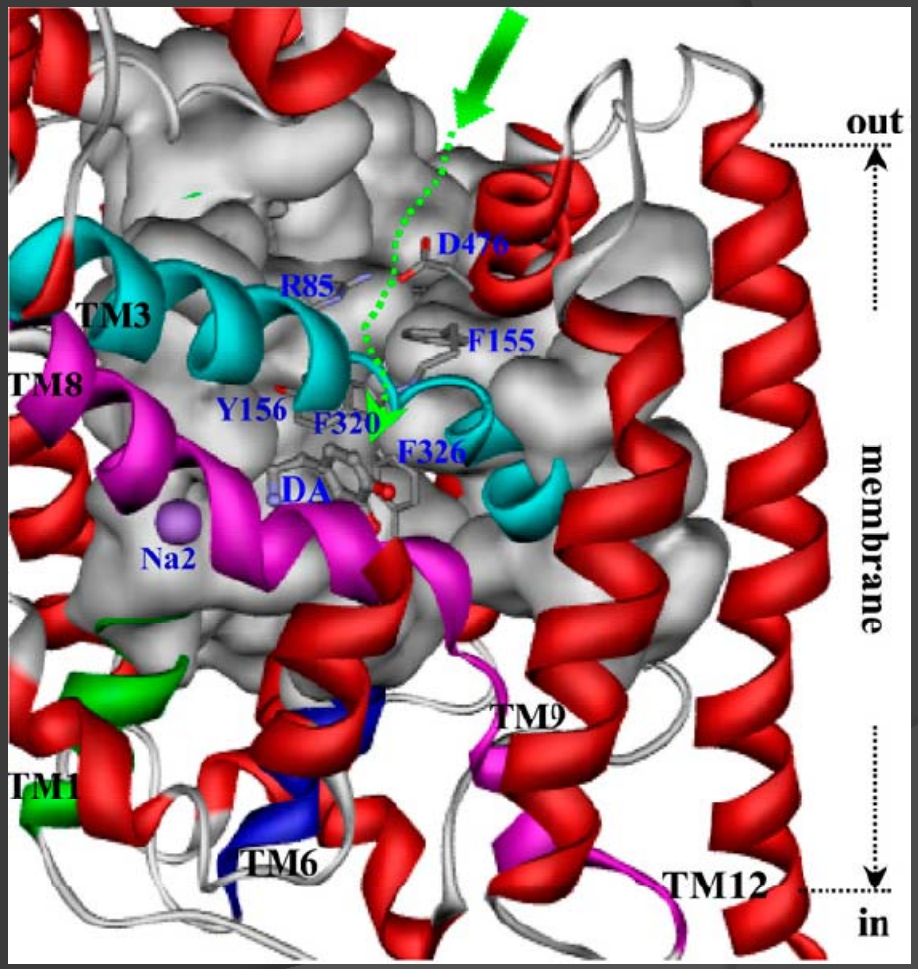
Comparison of ΔG_{bind} with Experiment

	Initial DAT complex	DAT complex during MM/MD calculations	Experiment
ΔG_{bind} (kcal/mol)	-5.6	-6.4	-7.4
K_d (μm)	78	20	---
K_M (μm)	---	---	3.466 ± 0.200

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