

Project title:

Atomic Resolution Description of the Transport Cycle in Neurotransmitter Transporters

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Executive Summary:

The human dopamine transporter (hDAT) belongs to the neurotransmitter:sodium symporter (NSS) family, which harness the electrochemical gradient of ions to actively transport neurotransmitters. hDAT-substrate coupling induces large-scale conformational changes between the outward-facing (OF) and inward-facing (IF) states, the details of which remain unknown. To characterize the key conformations of hDAT at the atomic level, as well as to structurally elucidate and thermodynamically characterize the transitions between them, we have employed all-atom molecular dynamics (MD) simulations along with enhanced sampling techniques. The results provide atomic details of the coupling between substrate-binding and the OF-IF transitions of the protein, demonstrating how substrate binding is strongly coupled to and modulates the OF-IF transition energy barrier.

Key Challenges:

In recent years, crystal structures of *Drosophila melanogaster* dopamine transporter (dDAT) [1,2] and human serotonin transporter (hSERT) [3] were solved in their outward-facing (OF) fully-bound states, shedding the first light on the atomic detail of neurotransmitter:sodium symporters (NSSs). However, the inward-facing (IF) structures are still missing, and the details of the OF-IF structural transition are unknown. Using Blue Waters, we attempted to bridge the gap between static crystal structures and dynamics conformational transitions of human dopamine transporter (hDAT) in different substrate-binding states by carrying out advanced molecular dynamics (MD) simulations.

Why it matters:

NSSs are membrane transporters that play an essential role in regulating neurotransmitter signaling and homeostasis. Malfunction of NSSs is connected to several neurological disorders including depression, Parkinson's disease, anxiety, attention deficit hyperactivity disorder, and epilepsy. Harnessing the transmembrane electrochemical gradient of ions, NSSs alternate between OF and inward-facing IF states to actively transport neurotransmitters through an alternating access mechanism. Detailed characterization of this process will enable a deeper understanding of the functional mechanism of this important family of transporters and help develop more effective pharmacological interventions for treatment of neurological disorders and drug addictions that are associated with NSSs.

Why Blue Waters:

Extended and multiple-copy simulations are keys to developing reliable quantitative results, e.g., reliable non-equilibrium work values and free energy profiles of specific steps involved in structural transitions of the protein. NAMD (NANoscale Molecular Dynamics) has been extensively optimized specifically for Blue Waters, showing sustained petascale performance enabling such calculations. Such calculations are only possible on platforms such as Blue Waters that provide platforms compatible with these demanding calculations.

Accomplishment:

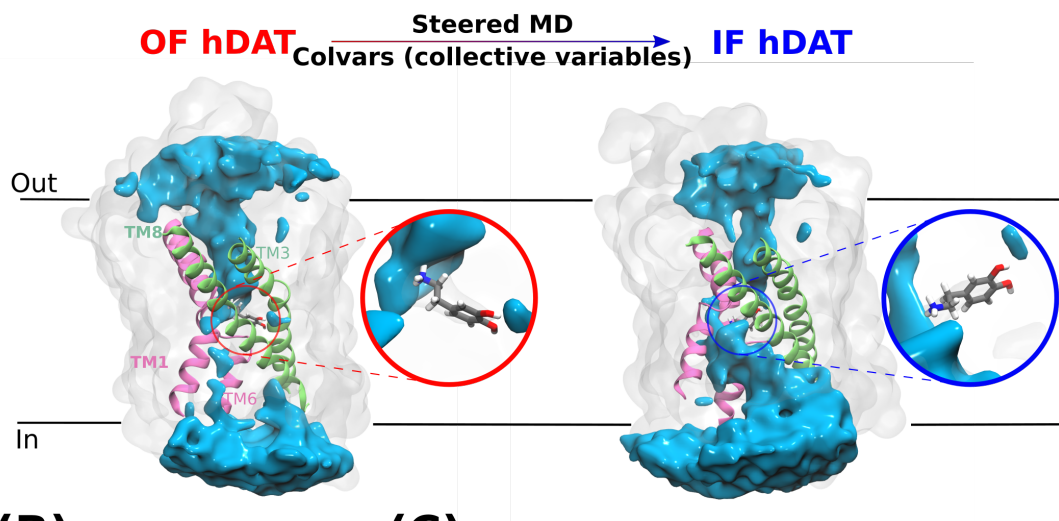
MD simulations provide a powerful tool to investigate biological macromolecular systems at the atomic level. However, the slow turnover rate of hDAT (around one substrate per second) makes it impractical to study the large-scale structural transition using conventional MD. To overcome this challenge, we employed knowledge-based biased MD simulations to describe large-scale conformational transitions using enhanced sampling techniques. We searched for collective variables (CVs) that best capture the global conformational differences between the OF and IF states, based on which we applied non-equilibrium, driven simulations to measure the non-equilibrium work to steer the protein to undergo the OF-IF transition while hDAT is in its (1) fully bound (OF_{holo}) state: bound to both the substrate (dopamine) and cotransported ions (two Na^+ and one Cl^-); (2) partially bound (OF_{pb}) state (bound only to ions); and, (3) the unbound (OF_{apo}) state. All simulations were carried out using NAMD (NANoscale Molecular Dynamics) with the CHARMM36m force field. Multiple simulations were performed for each biasing protocol.

A major outcome of the present modeling study was development of structural models for functional states of hDAT. Due to the lack of structural data on hDAT, an OF homology model of hDAT was generated using the crystal structure of *D. melanogaster* dopamine transporter (PDB ID: 4XP1) as a template. It was then equilibrated in membrane environment (at least 250 ns) in fully bound (OF_{holo}), partially bound (OF_{pb}), and *apo* (OF_{apo}) states in independent simulations. An initial IF homology model (IF_{ini}) of hDAT was built based on the membrane-equilibrated (13 μs) IF structure of its bacterial homologue, LeuT [4]. By driving four dopamine-binding helices in OF_{holo} to their coordinates in IF_{ini} using driven MD simulations, a more reliable IF_{holo} is obtained. Comparing IF_{holo} to OF_{holo} , we designed a variety of CVs from which we have determined the best CVs to capture the global conformational differences between OF and IF states. Namely, these CVs include the relative orientations between two pairs of transmembrane helices that expose the substrate-binding pocket of hDAT to the extracellular and the cytoplasmic space, respectively, and the distances between two pairs of ion-binding residues that control the opening of the ion binding pockets and block the substrate-binding pocket from the cytoplasmic side.

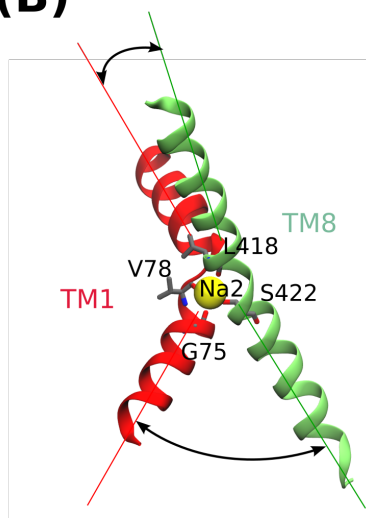
Following these CVs, we have steered the hDAT from OF_{holo} / OF_{pb} / OF_{apo} to their corresponding IF states in at least three independent runs (see, e.g., Figure 1). Comparing the non-equilibrium work, it was found that OF_{apo} requires minimum work to undergo the transition (79 ± 6

kcal/mol), OF_{pb} requires the most (103 ± 4 kcal/mol), while OF_{holo} requires an intermediate amount (96 ± 4 kcal/mol). This is fully consistent with the alternating access mechanism, according to which OF hDAT faces a lower energy barrier to alternate between the OF and IF states in its *apo* or fully-bound states. The simulations provide molecular details on the mechanism of this coupling. Trajectories connecting the two states provide a potential transition pathway that can now be experimentally tested in our collaborators' laboratory, e.g., by specific mutations of residues and measuring the function. These results provide the first molecular view of the transition mechanism in hDAT, paving the way to further free energy calculations and for rational drug screening protocols.

(A)



(B)



(C)

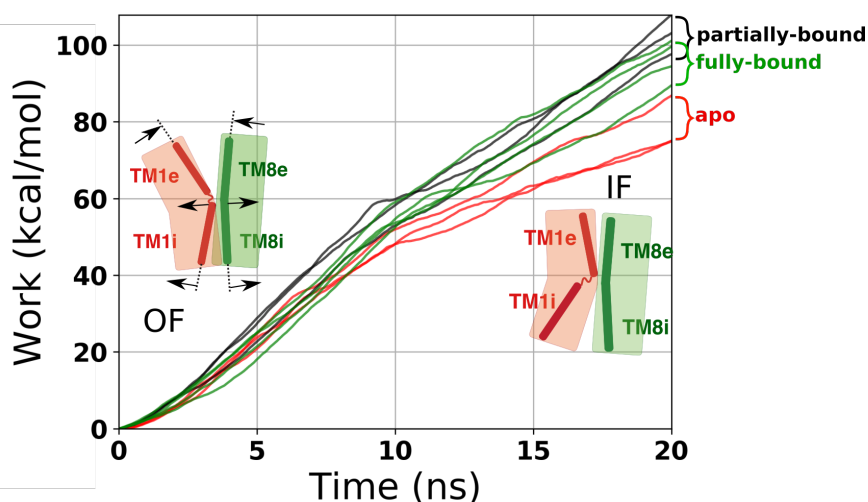


Figure 1: (A) Comparison of hDAT in OF and IF states. (B) The selected CVS, i.e., the relative orientation of TM1 and TM8 as well as the distances between ion binding residues. (C) Non-equilibrium work required in the simulations to drive hDAT from the OF to the IF state.

- [1] Penmatsa, A., K. H. Wang, and E. Gouaux. X-ray structure of dopamine transporter elucidates antidepressant mechanism. *Nature* 503.7474 (2013), 85.
- [2] Wang, Kevin H., A. Penmatsa, and E. Gouaux. Neurotransmitter and psychostimulant recognition by the dopamine transporter. *Nature* 521.7552 (2015), 322.
- [3] Coleman, Jonathan A., E. M. Green, and E. Gouaux. X-ray structures and mechanism of the human serotonin transporter. *Nature* 532.7599 (2016), 334.
- [4] Krishnamurthy, Harini, and E. Gouaux. X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. *Nature* 481.7382 (2012), 469.