SIMULATIONS UNCOVER THE MECHANISM OF SEROTONIN TRANSPORT IN THE BRAIN

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EXECUTIVE SUMMARY

The serotonin transporter (SERT) is a member of the neurotransmitter:sodium symporters (NSS) family that transports neurotransmitters in conjunction with an electrochemical gradient of ions. SERT initiates the reuptake of extracellular serotonin in the synapse to terminate neurotransmission in the nervous system. Recent cryogenic electron microscopy structures have revealed structural insights into functional conformations of SERT dynamics. However, despite being a major molecular drug target, knowledge of how serotonin is recognized, bound, and transported remains unclear. In this study, the research team performed extensive large-scale molecular dynamics (MD) simulations of the human SERT to investigate the structural transition to various states and determined the complete transport pathway in SERT. Further, the team provided a comprehensive approach for characterizing the thermodynamics of key states and critical residues involved in the substrate transport process.

RESEARCH CHALLENGE

The neurotransmitter serotonin (5HT) regulates many physiological processes in the body with implications for cognitive function, sleep, mood, and behavior [1]. In neurons, 5HT signaling is terminated through the reuptake of 5HT from the synaptic cleft by the SERT. Imbalance of 5HT in neurons owing to malfunctions of SERT has been associated with depression, bipolar disorder, and autism. Because of its medical importance, SERT is a major drug target for the treatment of psychiatric disorders and drugs of abuse. Selective serotonin reuptake inhibitors are commonly prescribed as antidepressants and aim to bind and inhibit SERT activity. However, these molecules can be responsible for a number of side effects. SERT and the closely related dopamine transporter (DAT) and norepinephrine transporter (NET) belong to a class of monoamine transporters. Owing to their high sequence and architecture similarities, it is difficult to design selective inhibitors to target just one transporter alone. Furthermore, these transporters undergo large conformational transitions from an outward-facing state to an inward-facing state to transport substrates across the cell membrane [2]. The lack of knowledge of the dynamics of these transporters has led to a lack of understanding of the molecular basis of selectivity for designing effective antidepressants and therapeutic molecules.

METHODS & CODES

Obtaining sufficient sampling is a recurring challenge in simulating complex biological processes. To overcome this issue, the research team adopted a Markov state model (MSM)-based adaptive sampling methodology to efficiently explore the conformational landscape. In each round of adaptive sampling, multiple short MD simulations were conducted in parallel. The simulation data were clustered using the K-means algorithm based on a designated metric and starting structures were chosen from the least populated states to seed the subsequent rounds of simulation. The simulations were employed using the AMBER v18 suite [3]. The highly parallelized framework implemented in AMBER offers enhanced scaling on GPU nodes to massively accelerate complex biomolecular simulations.

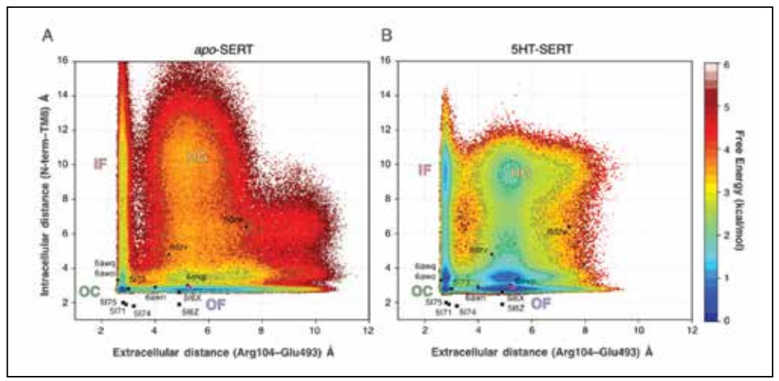

Figure 1: Conformational free energy landscapes of SERT obtained from MD simulations. Relative free energies from MSM-weighted simulation data plotted against the distances between extracellular and intracellular gating residues for (A) *apo*-SERT and (B) 5HT-SERT. The outward-facing SERT crystal structure (Protein Data Bank: 5I73, pink star) was used as the starting structure for MD simulations.

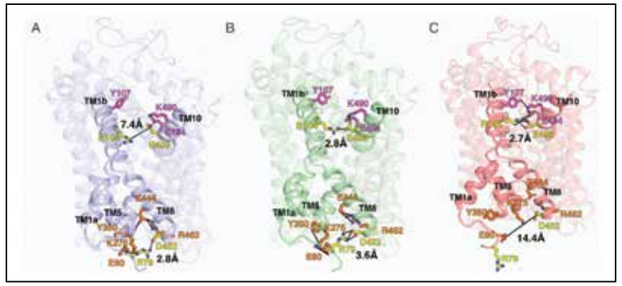

Figure 2: Switching of the hydrogen bond network drives structural transitions. MD snapshots of three conformational states in (A) outward-facing (OC), (B) occluded (OC), (C) inward-facing (IF). Residue interactions shown in sticks. Extracellular gating network colored in magenta; intracellular colored in orange. Gating residues colored in yellow.

RESULTS & IMPACT

Conformational dynamics of human SERT. To understand the effects of substrate-induced protein dynamics, the entire import process of serotonin was studied using MD simulations. Simulations were initiated from the outward-facing (OF) SERT crystal structure (Protein Data Bank: 5I73). An aggregated total of approximately 290 microseconds (μs) of SERT simulations were obtained and analyzed using MSM, which parses the simulation data into kinetically relevant states and calculates the transition probabilities between the states. MSM-weighted simulations data were projected onto a coordinate system defined by distances between the extracellular and intracellular gating residues (Fig. 1).

The conformational landscape plots reveal that despite the absence of serotonin binding, *apo*-SERT may undergo transitions from the OF state to the inward-facing (IF) state (Fig. 1a). Extracellular gating residues Arg104 (TM1b) and Glu493 (TM10) can extend to 10 Å, enlarging the extracellular entrance tunnel. The OF states are relatively stable, with a free energy of approximately 0.5 kcal/mol. The distance between gating residues Arg104–Glu493 decreases to 3 Å and is associated with electrostatic interactions, forming occluded (OC) conformations that are more stable than the OF state (Fig. 2). Closure of the extracellular entrance tunnel in the OF state weakens contacts on the intracellular side of the transporter, creating an energetically accessible pathway toward the IF state. The free energy barrier for transition from the OC–IF state in *apo*-SERT is estimated as approximately 3 kcal/mol, which is higher compared to the OF–OC transition (approximately 2 kcal/mol).

Serotonin transport in SERT. The substrate bound conformational landscape plot depicts deviations in the relative free energies of conformational states and reduced the free energy barriers in between states (Fig. 1b). Binding of serotonin in the entrance tunnel stabilizes the OF states. The gating residues interact with Gln332 (TM6) and Lys490 (TM10) and widens the extracellular vestibule (Fig. 2). The diffusion of 5HT to the S1 site via the allosteric site (S2) leads to the closure of the extracellular cavity to obtain the OC state. The OF–OC transition has a free energy barrier of approximately 1.5 kcal/mol, which is similar to *apo*-SERT. The downward movement of 5HT facilitates the opening of the intracellular gate and leads to the IF state. The free energy barrier for the structural transition to the IF state is estimated as approximately 1.5 kcal/mol. The presence of 5HT in the intracellular pathway stabilizes SERT in the IF state, with a free energy of approximately 1 kcal/mol as compared to approximately 3 kcal/mol in *apo*-SERT.

This study reveals an atomistic-level perspective into the elusive mechanism of substrate transport in SERT. Using MSM, the research team has identified the free energy barriers associated with the transport process and key interactions that drive transport. The results provide an extensive understanding into the molecular recognition of serotonin in SERT and can serve as a model to study other closely related neurotransmitter transporters.

WHY BLUE WATERS

Simulation of complex biological processes requires multiple parallel nodes to reach relevant timescales. The unique architecture of Blue Waters provides hybrid CPU and GPU frameworks to conduct large-scale simulations. These computations would not be achievable within a reasonable time without Blue Waters' petascale computing capability.

PUBLICATIONS & DATA SETS

M. C. Chan, B. Selvam, H. J. Young, E. Procko, and D. Shukla, "The substrate import mechanism of the human serotonin transporter," in preparation, 2019.

H. J. Young, B. Selvam, M. C. Chan, D. Shukla, and E. Procko, "Deep scanning mutagenesis reveals hotspot residues that alter conformational equilibrium in SERT," in preparation, 2019.

H. J. Young, B. Selvam, M. C. Chan, D. Shukla, and E. Procko, "Deep scanning mutagenesis reveals hotspot residues that alter conformational equilibrium in SERT," presented at the Gordon Research Conf. Mechanisms Membrane Transport, New London, NH, USA, June 23–28, 2019.

M. C. Chan, B. Selvam, and D. Shukla, "Substrate-induced conformational transitions of the human serotonin transporter," presented at the ACS Fall Nat. Meeting, San Diego, CA, USA., Aug. 25–29, 2019.