Neurotransmitter transporters (NTTs) are thought to play a key role in the etiology, symptoms, and/or palliative treatment of several neural disease states. They may be involved in such disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), attention deficit hyperactivity disorder (ADHD), substance abuse, depression, and epilepsy. Most mammalian NTTs belong to a super-family of Na+/Cl−-dependent transporters that contain twelve membrane-spanning domains. Understanding these proteins at the structural level not only allows basic research into the biophysical function of this large family of proteins, but also may help to guide ligand design for new and improved therapeutics and/or research tools.

Unfortunately, little to no empirically derived structural information, viz. X-ray crystallography or NMR spectroscopy, exists for the mammalian NTTs. The X-ray crystal structure of a related bacterial transporter for the amino acid leucine (LeuT) has been determined to 1.65Å resolution.1 Recently, the same group determined the structure of LeuT with leucine or alanine as well as various tricyclic antidepressants bound.2 Tricyclic antidepressants (TCAs) are promiscuous inhibitors of various NTTs and were found by Singh and colleagues3 to bind to and inhibit LeuT as well. Even though the average sequence identity between LeuT and the NTTs is around 18%, the proteins are believed to be homologous and thus share a significant degree of 3D structure similarity, especially within the trans-membrane regions.2 Beuming and colleagues4 warn, however, that even with a sufficient alignment, direct homology modeling is insufficient for the more variable regions, such as the internal/external loops. They further propose that additional structural information along with modeling and simulation will be required to get accurate models of the NTTs.

Various labs have developed homology models for NTTs based on the X-ray crystal structure of LeuT. Most of these models, however, are mainly based on the original, TCA-free LeuT structure. Seeing as most therapeutics that target NTTs are inhibitors, the TCA-bound LeuT structure may give us unique insights into possible binding sites for inhibitors of the NTTs. Moreover, only one group has reported performing molecular dynamics in a NTT homology model based on LeuT in a simulated membrane environment, although they used a very simplified membrane system.5 Due to these proteins having both aqueous and membrane-associated regions, they can be very computationally expensive to model accurately.

This poster details the results of using a TeraGrid Startup Allocation to run fully solvated 15 ns molecular dynamics simulations on a human norepinephrine transporter (hNET) homology model in an explicit membrane environment consisting of 170,317 atoms.

The full sequence hNET homology model was threaded based on the alanine-, clomipramine-, and sodium-bound X-ray crystal structure using the MODELLER software and its DOPE loop algorithms. Sequence alignments were adjusted based on previous simulated annealing alignments performed by our lab and others.7 Sodium atoms and crystal waters were maintained in the homology model, however only one sodium atom was placed by MODELLER. Clomipramine and alanine hetero-atoms were not maintained for the homology model generation. The hNET homology model was placed in a simulated membrane bi-layer hydrated, NaCl ions added, and initial periodic boundary conditions were evaluated using VMD8 (Figure 2).

Iterative energy minimization and molecular dynamics simulations were performed with decreasing positional constraints in order to: (1) melt lipid tails; (2) pack the lipid and water around the protein, while excluding water from the lipid bi-layer; (3) allow new side chain orientations to pack; and (4) briefly allow periodic boundary conditions to equilibrate. These simulations were performed on either NCSA’s "Abe" or U of I’s "Queen Bee" computational clusters via a TeraGrid Startup Allocation using the NAMD software package.9

Before running long duration molecular dynamics (equilibration) simulations on the fully solvated unconstrained protein, the system was benchmarked using NAMD to determine optimum job cost and performance for the same job limited to 30 minutes run-time with increasing number of nodes requested (Figure 3). It was found that 32 nodes (256 cores) was an ideal balance of cost (Service Units per step) and performance (steps per min).

Figure 1: Human norepinephrine transporter (hNET) full sequence homology model in a fully solvated, explicit POPC membrane environment before MD simulations.

Figure 2: Initial periodic boundary conditions of hNET homology model. Center cell shows full solvation, whereas other cells only show protein in lipid bi-layer before packing.

Figure 3: Benchmarking of NAMDs cost on NCSA’s "Abe" cluster. (A) Job Cost, here defined as: (SU/step vs. nodes allotted for a 30 minute wall-time job. Service Units (SU) are calculated by number of nodes x wall time and are the charge unit for a TeraGrid allocation. (B) Performance, here defined as number of steps completed in 30 minutes vs. nodes allotted in log, scale. Orange dashed line indicates perfect performance after doubling of performance units.

Figure 4: Bond lengths of sodium coordination and intramolecular hydrogen bonds within hNET homology model over the course of a 15 ns production run molecular dynamics simulation.

The fully solvated, unconstrained hNET homology protein inserted in a membrane bi-layer as a system of 170,317 atoms was equilibrated with periodic boundary conditions, constant number of atoms, constant pressure in the Z-direction, constant X-Y plane area, and constant temperature of 300K (NpT,AT ensemble) for 15 ns simulated molecular dynamics time. Protein stability and accuracy were monitored by evaluating bond lengths of residues believed to be involved in sodium coordination (Figure 4) and RMSD of protein backbone atoms (Figure 5). These methods are similar to the conditions used by Jørgensen and colleagues for their human serotonin transporter model.10

Most bond lengths stayed within 2-3Å, reasonable for an ionic or hydrogen bond. It is unknown why there is such a dramatic shift in bond lengths at about 7.5 ns for some residues. Backbone RMSD indicates that relative equilibrium has been reached by about 7.5 ns. These data seem support that these methods and the equilibrated model may be reasonable for further simulations, such as ligand docking.

Future efforts will focus on generating models with both sodium atoms bound, parameterizing known high-affinity ligands, and running molecular dynamics simulations with these ligands bound. Additional loop models may be attempted for regions lacking alignment to the LeuT structural template. Furthermore, models of other neurotransmitter transporters may be generated using similar methods. Finally, experience from this project will help to support research computing at the University of Montana as well as interest in and use of the TeraGrid.

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