Molecular Dynamics Simulations of LeuT Transport Mechanism Restrained by Normal Modes

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Abstract

Na$^+$/Cl$^-$-dependent transporters play key roles in driving the movement of neurotransmitters, such as dopamine and serotonin, across cellular membranes. The protein LeuT is a bacterial homologue of these transporters and allows for the selective diffusion of the substrate leucine through membranes in *Aquifex aeolicus*. Here, we aim to determine the plausibility of a previously proposed transport mechanism that involves a specific conformational change upon substrate/Na$^+$ transfer. We apply a molecular dynamics procedure that uses normal modes derived from anisotropic network modeling (ANM) to harmonically restrain the protein during simulation. Deformations along multiple frequency modes provide an efficient way to sample potential conformational changes in the presence of a water and membrane environment. ANM-restrained simulation is useful in determining a relatively low-energy transport pathway.
Background

Cells coated in a membrane, typically a phospholipid bilayer, which protects intracellular components from extracellular environment. Proteins embedded within the membrane allow for diffusion of select ions into the cell. The transport protein that will be studied is LeuT, a $\text{Na}^+ / \text{Cl}^-$-dependent transporter that catalyzes the thermodynamically unfavorable movement of its substrate, the amino acid leucine.

$\text{Na}^+ / \text{Cl}^-$-dependent transporters typically terminate synaptic transmission by using electrochemical gradients to drive the uptake of neurotransmitters from the synapse to the cytoplasm of neurons and glia. The dysfunction of these transporters has been linked to many diseases of the nervous system.

Figure from Engelman, DM (2005) Membranes are more mosaic than fluid. Nature 438, 578-580.
**Background**

LeuT promoter opens to the extracellular region, with the base facing the cytoplasm and ending 6Å into the bilayer-spanning part of the transporter. LeuT forms a dimer in the crystal, with the two promoters being parallel. However, only one LeuT protein is considered necessary for effective transport.

**Protein core**
- ten transmembrane segments
- leucine and two sodium ions found bound inside the protein core, halfway across the bilayer, in an area lacking water
- sodium ions have important roles in stabilizing the LeuT core and the bound leucine molecule
- a chloride ion was found bound on the outer surface of the protein and does not substantially affect transport capability.

The isolated LeuT structure had both intracellular and extracellular gates closed, but provided clues as to the transport mechanism. It is possible that binding and unbinding of substrate and ions to unwound joints may stabilize the α-helices in different conformational states. Ultimately, we aim to determine the validity of the proposed mechanism for the LeuT transport of leucine.
Protein Structure

LeuT Structure showing bound leucine (red) and two sodium ions (blue)
Proposed Mechanism

The proposed mechanism shows three major conformational states of the protein. Without a substrate and sodium ions, the LeuT assumes an outward-facing conformation. When the substrate enters the channel, the outward-facing opening partially closes. As the substrate and ions enter the cell, the outward-facing opening fully closes while the inward-facing residues assume an open conformation.
Simplifying the simulation:  
Anistropic Network Model

ANM-restrained MD provides a way to increase the efficiency of the simulations. Initially, the protein coordinate file is fed into ANM, which generates the 20 lowest modes. Of these modes, we choose the three or four that show the most collective motion. For each mode, we create two target conformations, a (+) one and a (-) one.

The two conformations differ only in the direction the protein is deformed along the eigenvector. Then a 20ps simulation is run while harmonically restraining the protein to a target conformation, after which the structure is minimized for at least 1000 steps. Thus for n modes, we will end up with 2n possible final conformers for the protein. Of these possible conformations, we choose the one lowest in energy. This process is repeated in 20ps steps until the protein reaches a reasonable end structure.
ANM uses elastic network methodology to represent the system at a residual level. The molecule is represented as a network, with each alpha-C as a node. The network calculates all interactions within a certain cutoff distance.
Other programs: VMD, NAMD

**VMD**

Visual Molecular Dynamics (VMD) is a molecular graphics program that allows for the display and examination of groups of molecules. VMD provides a way to visualize selections of atoms in multiple representations, view and analyze NAMD trajectory files, add water and membranes to the protein environment, and observe ANM modes.

**NAMD**

NAMD is a scalable molecular dynamics program that supports parallel processors. Molecular dynamics programs like NAMD simulate the behavior of biomolecular systems by computing interactive forces on an atomic scale. NAMD takes as input a protein structure file (tells connectivity between atoms), a protein coordinate file (gives the location of atoms in space), a topology file (tells atomic interactions) and a configuration file (for instructions and system information). NAMD computes atomic forces and movement at each timestep of 2fs, and ultimately outputs a trajectory file showing the system’s movement over time. A NAMD “run” can involve a minimization, whereby each step moves the system to a path of lower lower energy, an equilibration, where forces between atoms dictate the movement of the system, or a targeted simulation, where the system is driven towards a target structure.
Of the 20 lowest LeuT ANM vibrational modes, modes 4, 6, 13 and 19 were the most promising. They showed fairly widespread motion, as well as definite movement around the inward- and outward-facing parts of the protein and the protein channel.
Target Conformations

(-) Conformation

(+) Conformation
Results

"RMSD of protein vs. timestep" during initial 5000 step minimization of protein-water system with backbone fixed

We consider two systems: protein-water and protein-water-membrane. Each system is minimized with protein fixed for 5000 steps, equilibrated for 200ps, minimized with all atoms free for another 5000 steps, and equilibrated for 5ns.

We then run 20ps simulations on each system. So far, the results seem to indicate a good possibility that the proposed mechanism is close to the actual one. The motions of the protein are in the areas expected by the proposed mechanism. More simulations need to be done, however, to reach a definite conclusion.
Future Research

We will need to apply the ANM-restrained approach for more time steps, until the major outward-facing and inward-facing conformations are reached.

After we have simulated the full mechanism by which LeuT transports leucine, we can determine which conformational changes take place and what role the Na$^+$ ions play in transport. Beyond the exploration of LeuT, the final results of this experiment will likely allow for some insight into how other neurotransmitter transporters function. Possible applications include the dopamine and serotonin transporters. Both dopamine (a key hormone in a variety of animals) and serotonin (a neurotransmitter that regulates anger and aggression, among other things) play crucial roles in the human nervous system.
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References


Questions