CHARACTERIZING STRUCTURAL TRANSITIONS OF MEMBRANE TRANSPORT PROTEINS AT ATOMIC DETAIL

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EXECUTIVE SUMMARY:

Membrane transporters actively and selectively pump molecules in or out of the cell. The central role of these proteins in diverse physiological processes such as metabolism and neurotransmission makes them key drug targets. Their transport mechanisms often involve large-scale conformational changes coupled to the translocation of transported species such as ions, nutrients, and drugs. However, mechanistic details of these transport processes at an atomic level remain elusive due to the limitations of both experimental and computational techniques.

We have developed a novel computational approach to reconstruct such transport cycles using ensemble-based molecular dynamics simulations within an iterative framework involving high-dimensional path-finding algorithms and free-energy calculations. Employing Blue Waters resources and our novel non-equilibrium approach, the transport cycles of several membrane transporters have been reconstructed at an atomic level and characterized thermodynamically, providing an unprecedented level of detail about the transport mechanism.

INTRODUCTION

All living organisms rely on continuous exchange of diverse molecular species across cellular membranes for their function and survival. Membrane transporters are specialized molecular devices that provide the machinery for selective and efficient transport of materials across the membrane. They actively pump their substrates across the membrane by taking advantage of different forms of cellular energy. The biological and biomedical relevance of mechanistic studies on membrane transporter proteins cannot be overstated, given their central role in a myriad of key cellular processes and their involvement in the action of a vast number of pharmaceuticals. Large-scale conformational changes are central to the mechanism of membrane transporters. During the alternating-access mode of function [1], which is needed for uphill transport of the substrate against its electrochemical gradient, the protein switches substrate accessibility from one side of the membrane to the other. The process largely relies on large-scale structural transitions between two major states: the outward-facing (OF) and inward-facing (IF) states. Given the technical challenges involved in experimental characterization of these structural phenomena, simulation studies currently provide the only method to achieve the spatial and temporal resolutions required for a complete description of the transport cycle in membrane transporters, which by any measure constitute the most important aspect of the structural biology of these molecular machines.

METHODS & RESULTS

Large-scale structural transitions of membrane transporters cannot currently be characterized at an atomic level using conventional simulation technologies due to the long time scales involved (on the order of tens of milliseconds to seconds) [2]. Recognizing this issue, we recently developed a knowledge-based computational approach for describing large-scale conformational transitions using a combination of several distinct enhanced sampling techniques [3–5]. In the first stage, we used non-equilibrium, driven simulations based on system-specific reaction coordinates whose usefulness in inducing the transition of interest was examined using knowledge-based, qualitative assessments along with non-equilibrium work measurements. In the second stage, we use the string method with swarms-of-trajectories (SMwST) [5–7] in a high-dimensional collective variable space to further relax the optimized non-equilibrium trajectory obtained from the first stage and found an approximate minimum free-energy pathway. Finally, we used the relaxed trajectory to initiate bias-exchange umbrella sampling (BEUS) [3–5] simulations to estimate the free energies along the transition pathway.

We have studied the structural transition between the IF and OF states in several proteins from different classes of transporters including the ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp) and its bacterial homolog MsbA as well as major facilitator superfamily (MFS) transporters GlpT, GLUT1, and XylE using all-atom molecular dynamics (MD) simulations in the presence of an explicit membrane and solvent. In particular, we performed extensive non-equilibrium simulations to sample a large number of mechanistically distinct pathways for the conformational transition of MsbA [4].

The most relevant transition pathway identified using our approach was further investigated using free-energy calculations. The transition involved several distinct stages reflecting the complex nature of the structural changes associated with the function of the protein. The opening of the cytoplasmic gate during the outward- to inward-facing transition of apo (nucleotide-free) MsbA was found to be disfavored when the periplasmic gate was open and facilitated by a twisting motion of the nucleotide-binding domains that involved a dramatic change in their relative orientation. These simulations have been extended to study Pgp, a mammalian homolog of MsbA that is a multidrug resistance transporter (fig. 1).

In addition to ABC transporters, which use ATP hydrolysis as a source of energy, we have extensively studied several MFS transporters using our novel approach. Particularly, we have been able to provide a quantitative, atomic-level description of the functional thermodynamic cycle for the GlpT transporter by reconstructing the free-energy landscape governing the IF↔OF transition along a cyclic transition pathway involving both apo and substrate-bound states [5]. Our results provide a fully atomic description of the complete transport process, offering a structural model for the alternating-access mechanism and substantiating the close coupling between global structural transitions and local chemical events such as binding and unbinding of the substrate.

WHY BLUE WATERS?

Conventional MD simulations of large biomolecular systems suffer from poor conformational sampling, preventing one from achieving an accurate description of conformational ensembles and free-energy landscapes. The time scale limitation remains a great challenge, and the typical time scale of an atomistic MD simulation of biomolecular systems is much smaller than those required to describe most biologically relevant molecular phenomena. Some of the most powerful enhanced sampling techniques developed to address these issues rely on multiple-copy algorithms (MCAs) [7] that couple the dynamical evolution of a large number of copies of a system to explore the phase space and enhance the sampling. In our simulations, we employ BEUS and SMwST schemes, both of which are MCAs. Given that every copy of the simulation would require thousands of cores for its simulation, simulating a large number (typically hundreds) of interacting replicas simultaneously can only be accomplished on massive computing resources such as Blue Waters.

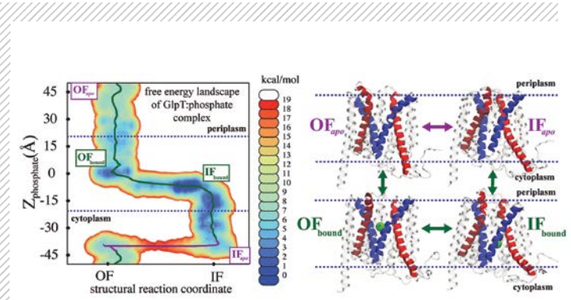

FIGURE 1: Transport cycle of MFS transporter GlpT characterized at an atomic level using enhanced sampling techniques and molecular dynamics simulations. The crystal structure of GlpT (IF_{apo} state) was used to reconstruct its entire transport cycle *(right)*. The transition pathway was then characterized energetically using 150 interacting replicas of GlpT system (each containing ~150,000 atoms) within the BEUS scheme *(left)*.

PUBLICATIONS

Li, J., P.-C. Wen, M. Moradi, and E. Tajkhorshid, Computational characterization of structural dynamics underlying function in active membrane transporters. *Curr. Opin. Struct. Biol.*, 31 (2015), 96–105, doi:10.1016/j.sbi.2015.04.001.

Moradi, M., G. Enkavi, and E. Tajkhorshid, Atomic-level characterization of transport cycle thermodynamics in a membrane transporter. *Nat. Commun.*, (accepted).

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