GLCPC Blue Waters Report

Project Title: Thermodynamic Characterization of Conformational Landscape in Proton-Coupled Oligopeptide Transporters

PI: Mahmoud Moradi, University of Arkansas, Fayetteville

Co-PI(s): None

Corresponding Author: Mahmoud Moradi, moradi@uark.edu

Executive Summary

Proton-coupled oligopeptide transporters (POTs) use the inwardly directed proton flow to uptake small peptides and peptide-like molecules. The human POT transporters PepT1 and PepT2 provide the main route through which the body absorbs and retains dietary proteins. Human POTs also recognize several important families of peptide-like drug compounds such as β-lactam antibiotics. In order to function, POTs undergo large-scale conformational changes, whose characterization is the key in understanding the mechanism of transport by these proteins. The inward- (IF) to outward-facing (OF) structural transition of POTs, however, has remained elusive despite much experimental and computational efforts. We have employed all-atom molecular dynamics (MD) simulations along with novel enhanced sampling techniques to, for the first time, characterize the large-scale conformational changes of a bacterial POT transporter, namely GkPOT. By employing novel loosely coupled multiple-copy (LCMC) algorithms, our simulations provide a detailed description of GkPOT conformational landscape, which sheds light on the structure-function relationship in POTs.

Key Challenges

Membrane transporters provide the machinery to intimately couple active transport of materials to various forms of cellular energy. POT transporters couple the energy from proton flow to the transport of dipeptides, tripeptides, and their analogs. A key feature of POTs is their substrate promiscuity, which is of great interest from a biomedical perspective. Human POT transporters PepT1 and PepT2, which play a key role in absorbing and retaining dietary proteins (in small intestine and kidney, respectively) recognize several important families of peptide-like drugs such as β-lactam antibiotics and can improve the uptake of poorly absorbed/retained medications if attached to amino acids or dipeptides (prodrugs). Recent structural studies have resulted in several crystal structures of bacterial POTs, among which GkPOT, the POT transporter found in the bacterium *Geobacillus kaustophilus*, has the highest resolution (1.9 Å). These crystal structures provide the basis of our understanding of POTs' transport mechanism at the structural level. These crystal structures are all in the IF state. However, in order to function as active transporters, POTs are known to alternate between distinct IF and OF states. The conformation of the OF state and the transition pathway between the two functional states have remained elusive.

Why It Matters

Previous MD simulations that have relied on equilibrium simulations have failed to reliably characterize large-scale conformational changes such as those involved in POTs. While the conventional MD can provide information on local conformational changes of a protein upon binding or unbinding of a substrate, ion, or proton, the global conformational changes observed are not often statistically significant. Functionally important conformational changes such as IF-OF transition in membrane transporters typically occur on timescales beyond those accessible to conventional all-atom MD. The large-scale conformational changes, on the other hand, are typically studied using simplified modeling techniques such as coarse-graining, which could completely ignore or misrepresent the role of chemical events in the transport process. The main challenge in characterizing the large-scale conformational changes of proteins such as those associated with GkPOT is to reach the functionally relevant timescales without compromising the chemical details. The successful employment of the cutting-edge LCMC algorithms implemented here using Blue Waters resources will open a new window to structural biology of membrane transporters, that bypasses the limitations of computational approaches to study structure-function relationships in these proteins. Unfortunately, the simulations were not finished as only half of the requested allocations were granted in the first submission and the renewal project was not accepted. We will continue the project once we can secure some allocation on Blue Waters in the future.

Why Blue Waters?

We have explicitly shown that the unbiased all-atom MD, which is routinely used in the field, could be quite misleading in deciphering mechanistic features of membrane transporters due to the great gap in the timescales associated with the conventional simulations and the function of these proteins (Immadisetty, et al, 2017). On the other hand, we have shown that the LCMC algorithms, e.g., BEUS/SMwST, can be used to reconstruct the unknown structural transitions of membrane transporters. Unlike the conventional all-atom or corase-grained MD that can be performed on sub-petascale machines, LCMC MD simulations are suited only for petascale machines such as Blue Waters since they require hundreds of nodes for a single job. We note that the "weak scaling" of these algorithms (Fig. 1) particularly makes them attractive for large petascale machines, as they can utilize hundreds of compute nodes with almost perfect efficiency. The software engine used for the simulations is NAMD. Multiple concurrent NAMD instances are launched by low-level point-to-point

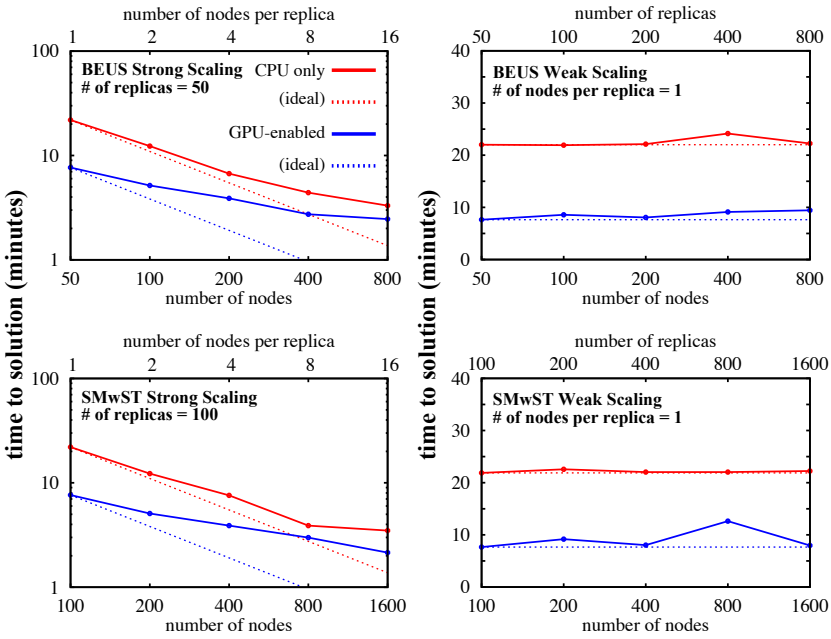

Figure 1 *Strong and weak scaling of the Riemannian BEUS and SMwST algorithms implemented in NAMD 2.10. Time to solution is reported for 40 ps of GkPOT simulations in explicit membrane and water (~85,000 atoms).*

communication functions, which are accessible through NAMD's Tcl scripting interface. This allows for the modification of the LCMC algorithms without the need to recompile the NAMD code.

Accomplishments

Here we have used a novel ensemble-based simulation approach to reconstruct the entire transport cycle of GkPOT. Bias-Exchange Umbrella Sampling (BEUS) and String Method with Swarms of Trajectories (SMwST) are two independent LCMC algorithms employed. Both methods require parallel execution of hundreds of MD simulations of large protein systems with explicit representation of water and membrane, which requires hundreds of nodes. This methodology, which efficiently takes advantage of petascale computing, is a rigorous and practical approach in characterizing large-scale conformational changes of proteins and their coupling to chemical events. An important modification to the methodology to increase the accuracy of the results was introduced that involves a Riemannian formulation of free energy calculation and path-finding algorithms. The OF structure shown in Fig. 2 represents the first OF model of POT transporters, generated using our all-atom MD simulations in combination with LCMC algorithms discussed above. Our model is verifiably a stable OF structure since the subsequent equilibrium simulations show a water accessibility consistent with an OF state (see the water profiles in Fig. 2). Our simulations also suggest that the full IF-OF transition requires the binding of both proton and substrate (data not shown). The pathways generated using parallel SMwST and modified using our recently introduced Riemannian formalism reveal that the proton-bound GkPOT cannot transition to the OF state (data not shown). Unlike the common simulation studies, which either rely on unbiased equilibrium simulations or simple representations (e.g., coarse-graining), our approach combines the accuracy of all-atom MD with the accessibility of long timescales provided by enhanced sampling techniques. While we have generated all the pathways required to reconstruct the transport cycle of GkPOT, we could not complete the BEUS simulations with the resources provided. Unfortunately, the project was not renewed and currently we cannot publish the data until we finish the simulations.

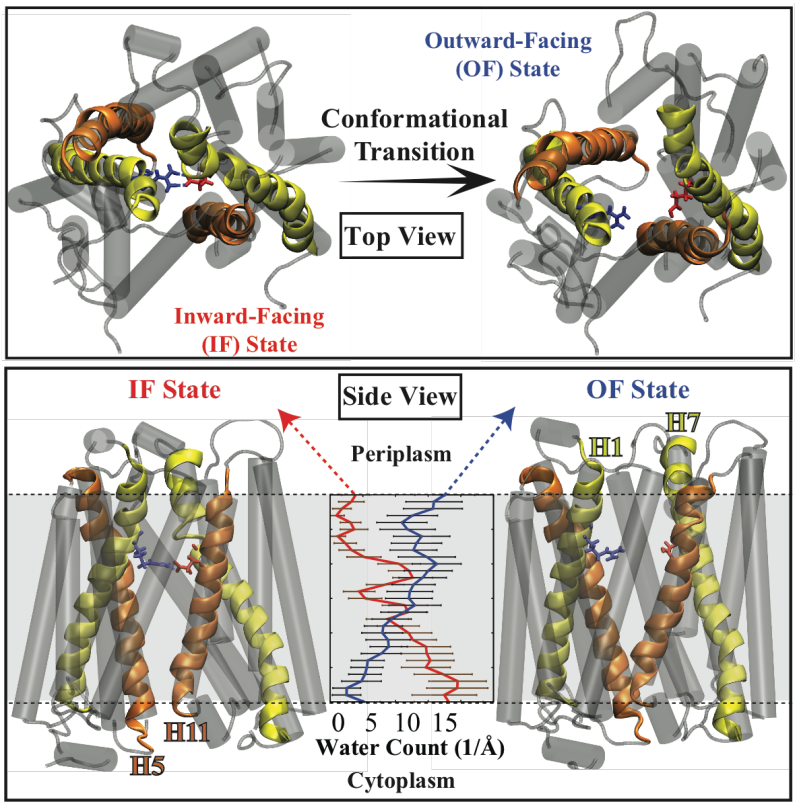

Figure 2 *Top and side views of GkPOT transporter (cartoon representation) in its IF state (PDB: 4IKV) and OF state (our model generated using enhanced sampling techniques), along with the water count along the pore as measured from equilibrium simulations of GkPOT.*

Publications

Immadisetty, K.; J. Hettige, and M. Moradi. What can and cannot be learned from molecular dynamics simulations of bacterial proton-coupled oligopeptide transporter GkPOT? *J. Phys. Chem. B.*, 121:15 (2017), pp. 3644–3656. **DOI: 10.1021/acs.jpcb.6b09733**

Moradi, M., K. Immadisetty, and J. Hettige, Couplings between local and global conformational changes in proton-coupled oligopeptide transporters. Biophysical Society Meeting, New Orleans, LA, February 11-15, 2017 (poster).

Immadisetty, K.; J. Hettige, and M. Moradi. Thermodynamic characterization of full transport cycle for a bacterial proton-coupled oligopeptide transporter. *In preparation. [Note: Cannot be published without completing the simulations]*