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TRANSPORT MECHANISM OF POT TRANSPORTERS: EMPLOYING LOOSELY COUPLED MOLECULAR DYNAMICS SIMULATIONS TO CHARACTERIZE PROTEIN STRUCTURAL DYNAMICS

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EXECUTIVE SUMMARY

Proton-coupled oligopeptide transporters (POTs) use the inwardly directed proton flow to uptake small peptides and peptidelike 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 peptidelike drug compounds such as β-lactam antibiotics. POTs undergo large-scale conformational changes that are the key in understanding the transport mechanism of these proteins. Despite many experimental and computational efforts, however, the inward- (IF) to outward-facing (OF) structural transition of POTs has remained elusive owing to limitations in methodology. Therefore, the researcher has employed novel molecular dynamics (MD)-based enhanced sampling techniques to characterize the large-scale conformational changes of a bacterial POT transporter, namely GkPOT. With the help of petascale supercomputing, these MD-based techniques provide a detailed description of GkPOT's conformational landscape, which sheds light on the structure–function relationship in POT transporters.

RESEARCH CHALLENGE

Membrane transporters provide the machinery to couple active transport of materials to various forms of cellular energy. POT transporters couple the energy from proton flow to the transport of small peptides and peptidelike molecules [1]. A key feature of POTs is their substrate promiscuity [2], 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 [3], recognize several important families of peptidelike drugs such as β-lactam antibiotics [4]. These proteins can uptake poorly absorbed/retained drugs when attached to amino acids or dipeptides as in prodrugs [5].

Recent structural studies have resulted in several crystal structures of bacterial POTs [6–9] such as GkPOT from *Geobacillus kaustophilus* [10]. These crystal structures, which are all in the IF state, provide the basis of our understanding of POTs' transport mechanism at the structural level. However, in line with the alternating access mechanism, POTs alternate between two distinct states; *i.e.*, the IF and OF states. The conformation of the OF state and the transition pathway between the two functional states have not been experimentally characterized.

Previous equilibrium MD simulations have failed to characterize large-scale conformational changes of POTs [10]. While 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 [11]. Functionally important conformational changes such as the 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 usually 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.

METHODS & CODES

The PI has used a novel ensemble-based simulation approach [12–15] to reconstruct the entire transport cycle of GkPOT. Bias-exchange umbrella sampling (BEUS) and string method with swarms of trajectories (SMwST) are both loosely coupled MD-based algorithms that require parallel execution of hundreds of MD simulations [14] and have recently been modified within a Riemannian geometry framework [15]. The methodology is specifically based on applying orientation-based forces on protein transmembrane helices in order to speed up the exploration of protein conformational space.

The software engine used for the simulations is NAMD, a highly scalable MD code implemented in Charm++, an object-based message-driven execution system based on C++. NAMD has been enhanced to support extremely scalable loosely coupled multiple-copy algorithms. Multiple concurrent NAMD instances are launched with internal partitions of Charm++ and located continuously within a single communication world. Messages between NAMD instances are passed by low-level point-to-point communication functions, which are accessible through NAMD's Tcl scripting interface.

RESULTS & IMPACT

The OF structure shown in Fig. 1 represents the first OF model of POT transporters and was generated using the researcher's

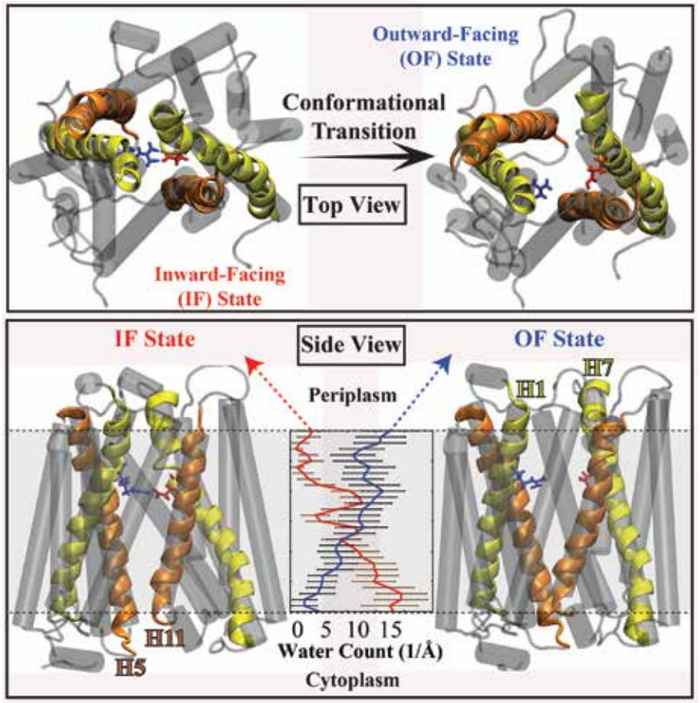

Figure 1: Top and side views of the GkPOT transporter (cartoon representation) in its inward-facing (IF) and outward-facing (OF) states, along with the water count along the pore as measured from equilibrium simulations of each model. The OF model was generated using the orientation-based enhanced sampling techniques.

all-atom MD simulations in combination with orientation-based BEUS/SMwST algorithms [16]. The model is verifiably a stable OF structure since the subsequent equilibrium simulations show a water accessibility consistent with an OF state (Fig. 1). The simulations also suggest that the full IF–OF transition requires the binding of both proton and substrate. The pathways generated reveal that the proton-bound GkPOT cannot transition to the OF state [16]. Unlike previous simulation studies, which had relied on either unbiased equilibrium simulations or simple representations (*e.g.*, coarse-graining), this new approach combines the accuracy of all-atom MD with the accessibility of long timescales provided by enhanced sampling techniques. The successful employment of these multiple-copy algorithms using Blue Waters resources opens a new window to the structural biology of membrane transporters that bypasses the limitations of computational approaches to studying structure–function relationships in these proteins.

WHY BLUE WATERS

This work has 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 owing to the great gap in the timescales associated with the conventional simulations and the function of these proteins [11]. On the other hand, loosely coupled multiple-copy algorithms such as BEUS and SMwST [14,15] can be used to reconstruct unknown conformational transitions of membrane transport proteins. Unlike the conventional all-atom or coarse-grained MD that can be performed on subpetascale machines, BEUS/SMwST simulations of membrane transporters are well-suited for large petascale computational resources such as Blue Waters since they require hundreds of nodes for a single job. The "weak scaling" of these algorithms makes them particularly attractive for large petascale machines, as they can utilize hundreds of compute nodes with almost perfect efficiency.

PUBLICATIONS & DATA SETS

K. Immadisetty, 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.*, vol. 121, no. 15, pp. 3644–3656, 2017, doi: 10.1021/acs.jpcb.6b09733.

D. Ogden, K. Immadisetty, and M. Moradi, "Conformational transition pathways in major facilitator superfamily transporters," *bioRxiv* 708289, 2019, doi: 10.1101/708289.