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ACTIVATION MECHANISMS OF THE MECHANOSENSITIVE CHANNEL OF LARGE CONDUCTANCE: EMPLOYING LOOSELY COUPLED MOLECULAR DYNAMICS SIMULATIONS TO CHARACTERIZE PROTEIN STRUCTURAL DYNAMICS

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EXECUTIVE SUMMARY

The mechanosensitive channel of large conductance (MscL) is a model system for the study of mechanosensation. Understanding MscL conformational dynamics has specific biomedical applications. The high level of conservation of MscL in bacteria and its absence from the human and animal genomes make MscL an attractive drug target for novel antibiotics. In addition, MscL has been proposed as a liposomal drug delivery nanovalve. For example, through engineering MscL can become pH-activated to release drugs when it senses the low pH of the tumor microenvironment.

The PI employed all-atom molecular dynamics (MD) simulations along with novel enhanced sampling techniques to characterize the large-scale conformational changes of MscL and its interactions with its candidate modulators. These simulations elucidate the conformational landscape of MscL at the molecular level, providing a rational design framework for designing more efficient modulators for MscL.

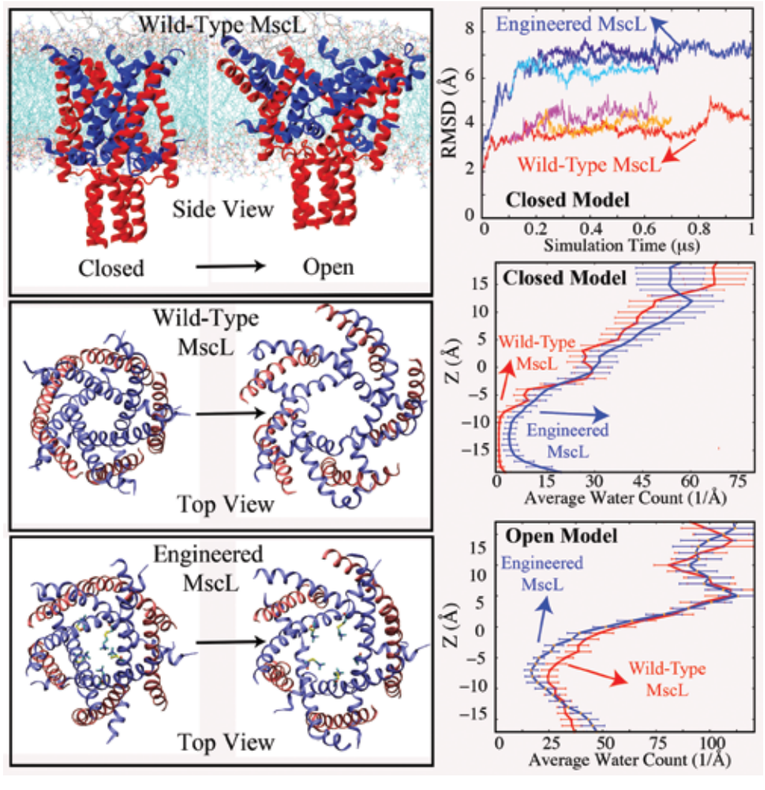

Figure 1: MscL protein (cartoon representation) in its closed (inactive) and open (active) states. (Left panels) In its wild-type (top and middle left) and engineered (bottom left) forms. (Right panels) The root-mean-square deviation (RMSD) time series (top right) and water profile along the pore (middle and bottom right) from various simulations.

RESEARCH CHALLENGE

The mechanosensitive channel of large conductance (MscL) [1] is a bacterial membrane transport protein that serves as a model system for the study of mechanosensation, a process involved in hearing, touch, balance, and cardiovascular and kidney regulation [2,3]. Owing to its unique properties, MscL has also been proposed for use in various biomedical applications, both as a drug target for novel antibiotics [4] and as a stimulus-triggered nanovalve for drug delivery liposomes [5–8]). Unfortunately, MscL structure is only known in its inactive, closed state [9]. If the molecular basis of MscL activation were understood, researchers could engineer more efficient functional nanovalves and design more potent antibiotics targeting MscL.

It is vital to model the active, open state of MscL as well as the entire opening/closing process in order to characterize the MscL activation mechanism. The timescales involved in this process are beyond the currently accessible limits of traditional all-atom MD. The tilting of transmembrane helices is the main rate-limiting step required to form the open pore [10]. While many MD studies have been conducted to study MscL activation, the techniques used in these studies rely on simplifications such as coarse-graining [11]. The main challenge in characterizing the large-scale conformational changes of proteins such as those associated with MscL is to reach the functionally relevant timescales without compromising the chemical details.

METHODS & CODES

The PI used a novel ensemble-based simulation approach [12–15] to simulate the activation process of wild-type and engineered MscL. 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 been recently modified within a Riemannian geometry framework [15]. The methodology is specifically centered 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 were launched with internal partitions of Charm++ and located continuously within a single communication world. Messages between NAMD instances were passed by low-level point-to-point communication functions, which are accessible through NAMD's Tcl scripting interface.

RESULTS & IMPACT

Using the known closed/inactive structure of MscL (Protein Data Bank: 2OAR [9]) and the enhanced sampling MD simulations conducted on an orientation-based biasing protocol, the PI has successfully generated an open model of MscL both for the wild-type and engineered protein (Fig. 1). The engineered MscL is made by attaching positively charged MTSET ([2-(trimethylammonium)ethyl] methane thiosulfonate bromide) labels to the protein within its gate region. The opening of the wild-type MscL is triggered by an increase in the membrane tension, whereas for the engineered MscL, the opening could occur spontaneously. Microsecond-level equilibrium simulations reveal the beginning stages of the activation of the engineered MscL (Fig. 1); however, enhanced sampling techniques are required to capture the complete activation (Fig. 1). Unlike previous simulation studies that relied on either unbiased equilibrium simulations or simple representations (*e.g.*, coarse-graining), the PI's approach combines the accuracy of all-atom MD with the accessibility of long timescales provided by enhanced sampling techniques.

The main conclusion of this project's simulations is that the wild-type and engineered MscL are associated with distinct open conformations. The knowledge of the details of the open state of MscL and its activation mechanism allows for designing more efficient modulators for the engineered MscL within a rational design framework. The successful employment of these multiple-copy algorithms using Blue Waters' resources opens a new window to the structural biology of membrane transport proteins that bypasses the limitations of computational approaches to studying structure–function relationships in these proteins.

WHY BLUE WATERS

The PI has explicitly shown that 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. 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 as they require hundreds of nodes for a single job. The "weak scaling" of these algorithms in particular makes them attractive for large petascale machines, as they can utilize hundreds of compute nodes with almost perfect efficiency.

PUBLICATIONS & DATA SETS

K. Immadisetty, A. Polasa, R. Shelton, and M. Moradi, "Elucidating the molecular basis of pH activation of an engineered mechanosensitive channel," *bioRxiv* 707794, 2019, doi: 10.1101/707794.