BLUE WATERS

SUSTAINED PETASCALE IN ACTION:
ENABLING TRANSFORMATIVE RESEARCH

2019 ANNUAL REPORT

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MICROSCOPIC IDENTIFICATION OF PIP_2 BINDING SITES ON A CA^{2+}-ACTIVATED CL^- CHANNEL

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EXECUTIVE SUMMARY

Membrane proteins dwell in a sea of phospholipids that not only structurally stabilize the proteins by providing a hydrophobic environment but also dynamically regulate protein function. While many cation channels are known to be regulated by the negatively charged phosphatidylinositol 4,5-bisphosphate (PIP_2), relatively little is known about anion channel regulation by phosphoinositides. Using atomistic molecular dynamics simulations on Blue Waters combined with experimental patch clamp electrophysiology, the research team has identified several PIP_2 binding sites in TMEM16A, a Cl^- (chloride) channel that performs myriad physiological functions ranging from epithelial fluid secretion to regulation of electrical excitability. These PIP_2 binding sites form a band at the cytosolic interface of the membrane that the team proposes constitutes a network to dynamically regulate this extensively allosterically regulated protein. The microscopic description of the PIP_2–TMEM16A interactions provided by this research adds a crucial layer of information for understanding the regulation mechanisms of ion channels by specific lipids.

RESEARCH CHALLENGE

TMEM16A is a Ca^{2+}-activated Cl^- channel that regulates diverse cellular functions including fluid secretion, neuronal excitability, and smooth muscle contraction [1–3]. The channel is activated by elevation of cytosolic Ca^{2+} and modulated by the anionic lipid PIP_2. PIP_2 is known to be an important signaling lipid and critical for the regulation of a wide variety of ion channels [4–7]. Thus, understanding the mechanism by which PIP_2 binds to and affects membrane channels is of broad physiological and biophysical relevance. Although previous experimental studies have illustrated the regulatory role of PIP_2 on TMEM16A [8–10], it remains elusive how PIP_2 interacts and binds to the channel and how the binding affects molecular events underlying transport in the protein. To understand the regulatory mechanisms of TMEM16A by PIP_2, it is important to identify amino acids involved in the lipid–protein interactions and characterize the conformational changes induced by these interactions. The obtained knowledge will help to unravel the atomic-level details underlying the functional mechanism of this highly allosteric protein and provide insight into the understanding of the mechanistically complicated TMEM16 superfamily.

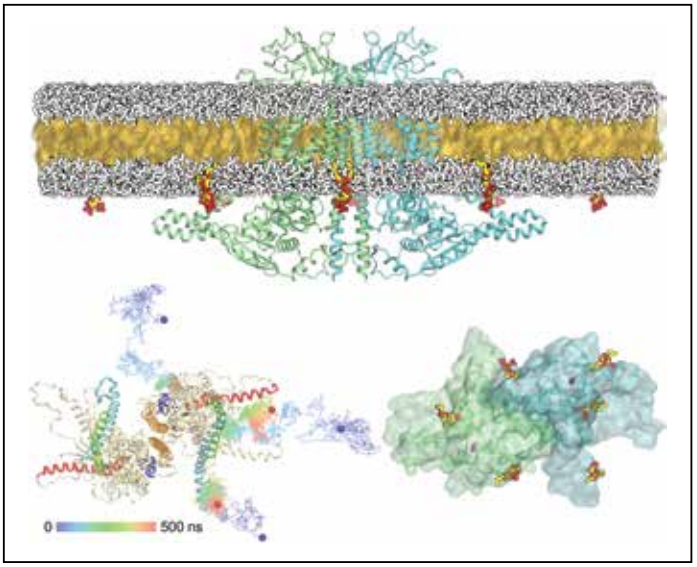

Figure 1: The initial simulation system viewed from the membrane, in which a fraction of the acyl tails of the membrane-forming lipids is replaced by a liquid organic phase to enhance the lipid diffusion. (Bottom) Top views showing representative PIP_2 binding trajectories (left) and binding positions (right).

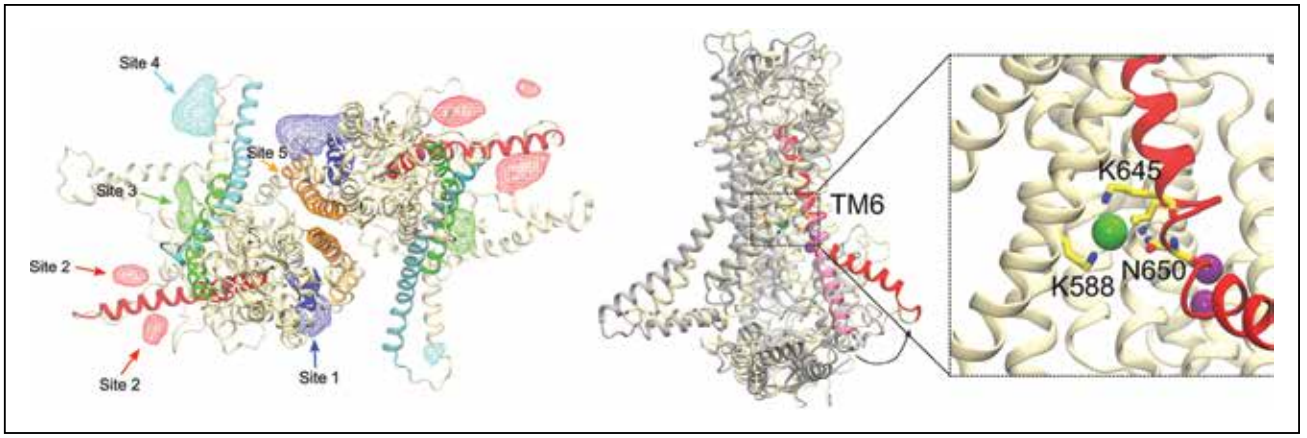

Figure 2: Maps of PIP_2 occupancy extracted from the simulations illustrate multiple PIP_2 binding sites near the functionally important transmembrane helices (individually colored). (Right) Representative snapshot showing the TM6 (transmembrane helix 6) conformational change in the presence of PIP_2. The dilation of the pore allows Cl^- (green sphere) to penetrate spontaneously.

METHODS & CODES

To gain insight into the binding of PIP_2 to TMEM16A, extended molecular dynamics (MD) simulations were performed on the atomic model of the ion channel [11] using the highly mobile membrane mimetic model (HMMM) [12]. The HMMM model was introduced to accelerate lipid diffusion in atomistic simulations in order to obtain enhanced sampling of the interaction of lipid headgroups with proteins within simulation timescales currently achievable. This model replaces a portion of the membrane hydrophobic core by a more fluid representation using simple carbon solvent ethane (SCSE), while employing short-tailed lipids to maintain a full description of the headgroups and the initial part of the tails (Fig. 1). This model provides a more flexible and mobile environment that allows for rapid rearrangement and displacement of the lipid headgroups, thereby facilitating phenomena that might be inaccessible with conventional membrane models owing to the inherently slow dynamics of the lipids. In each of the six independent simulation systems, eight PIP_2 molecules were added to the inner leaflet of an otherwise phosphatidylcholine (POPC) lipid bilayer evenly surrounding the protein at the beginning of the simulations. To determine whether binding of PIP_2 was influenced by full-length acyl chains, after the completion of lipid-binding simulations with HMMM membranes (500 nanoseconds [ns] each), short-tailed lipid molecules were converted back to full-length lipids, and the resulting full systems were subjected to additional equilibrium simulations of 100 ns each. All MD simulations were carried out on Blue Waters using the NAnoscale Molecular Dynamics (NAMD) simulation package [13].

RESULTS & IMPACT

The research team's unbiased atomistic MD simulations with approximately 1.4% PIP_2 in POPC bilayers revealed spontaneous binding of PIP_2 to several potential sites on the surface of the TMEM16A channel (Fig. 1). Three of these sites captured 85% of all PIP_2–protein interactions and were validated to be critical for PIP_2 regulation through mutagenesis experiments by the collaborators. Simulations showed that PIP_2 is stabilized by hydrogen bonding between basic residues and the phosphate/hydroxyl groups on the inositol ring of the lipid headgroup. Binding of PIP_2 to different sites produces different conformational effects in the cytoplasmic part of transmembrane helix 6 (TM6), which forms one side of the channel pore and plays a key role in channel gating. The occupation of the major sites is especially shown to induce a dramatic rotation of the cytoplasmic end of TM6 away from the pore (Fig. 2). This pore dilation increases the accessibility of the inner vestibule of the channel to the cytosolic ions and resulted in spontaneous penetration of Cl^- ions into the pore (Fig. 2). Based on this observation, the research team proposed that a network of PIP_2 binding sites at the cytoplasmic face of the membrane allosterically regulates channel gating. The data provided by these simulations add to a growing body of knowledge showing that TMEM16A is a highly allosteric protein that is gated by a network of interactions involving both Ca^{2+} and PIP_2.

WHY BLUE WATERS

The state-of-the-art architecture of Blue Waters makes it an excellent computing resource for this scientific research. The GPU-optimized simulation package NAMD has been extensively tested and optimized for Blue Waters. The large number of GPUs available on the XK nodes significantly increased the overall productivity. In addition, the technical support provided by the experts and scientists of the Blue Waters team has contributed to the accomplishment of the research goals by smoothing out technical issues that have arisen during the allocation.

PUBLICATIONS & DATA SETS

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