MOLECULAR MECHANISM OF LIPID AND ION TRANSPORT IN PHOSPHOLIPID SCRAMBLING

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EXECUTIVE SUMMARY

From bacteria to mammals, different phospholipid species are segregated between the inner and outer leaflets of the cellular membrane by adenosine triphosphate (ATP)-dependent lipid transporters. Disruption of this segregation by ATP-independent phospholipid scrambling is a key step in cellular signaling, e.g., inducing programmed cell death and blood coagulation. The mechanism by which scramblase (the protein responsible for phospholipid translocation) catalyzes rapid exchange of lipids between the two leaflets of a bilayer has been long sought. Using extensive molecular dynamics (MD) simulations on Blue Waters, we showed that a hydrophilic track formed on the surface of a scramblase serves as the pathway for both lipid and ion translocations, and that Ca^{2+} ion binding controls the open/closed transition of the track. This microscopic view of the lipid transport process sheds light on how lipophilic molecules can permeate specialized proteins to travel between the two leaflets of the cellular membrane—a process that is of broad physiological and biomedical relevance.

RESEARCH CHALLENGE

Different phospholipid species are distributed asymmetrically between the two leaflets of the cellular membrane. Dissipation of this asymmetry in response to the elevation of cytoplasmic Ca^{2+} concentration is a ubiquitous signaling mechanism critical for diverse cellular events including blood coagulation, bone mineralization, and cell–cell interaction [1–3]. Phospholipid scrambling is mediated by phospholipid scramblases, which harvest the energy of the phospholipid gradient to drive nonspecific and bidirectional transport of phospholipids between leaflets. Proteins responsible for Ca^{2+}-activated lipid scrambling belong to the TMEM16 superfamily of membrane proteins, with some members being Ca^{2+}-activated Cl– channels, while others function as Ca^{2+}-activated scramblases and nonselective ion channels. Despite the remarkably diverse functions of TMEM16 proteins, both subfamilies share a common dimeric architecture and mode of Ca^{2+} activation [4–6]. However, the absence of phospholipids and ionic substrates in the solved structures leaves the question of how both lipids and ions are conducted unanswered. It also remains elusive how the same architecture accommodates such diverse functions.

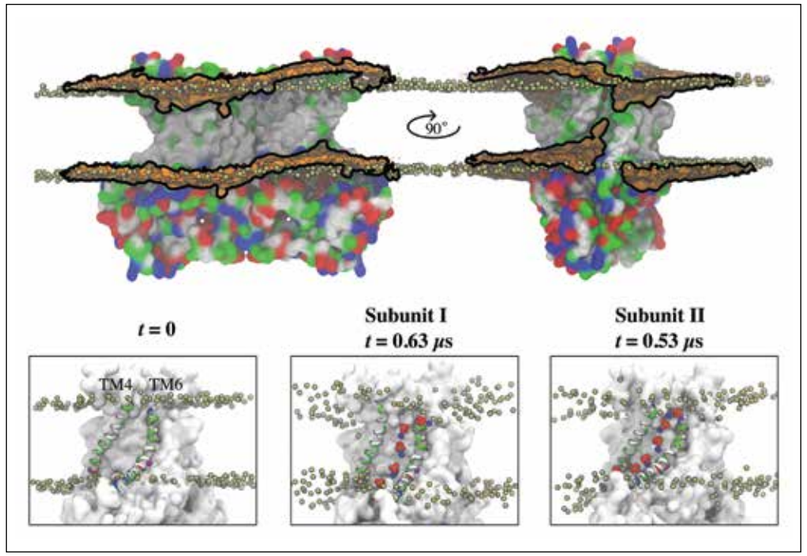

Figure 1: (Top) Membrane deformation induced by nhTMEM16, shown by the average phospholipid phosphate density over the simulation (orange surface) compared to the initial distribution (tan spheres). (Bottom) Representative simulation snapshots showing the empty track at t = 0, which becomes fully occupied by lipids in both subunits during the simulation.

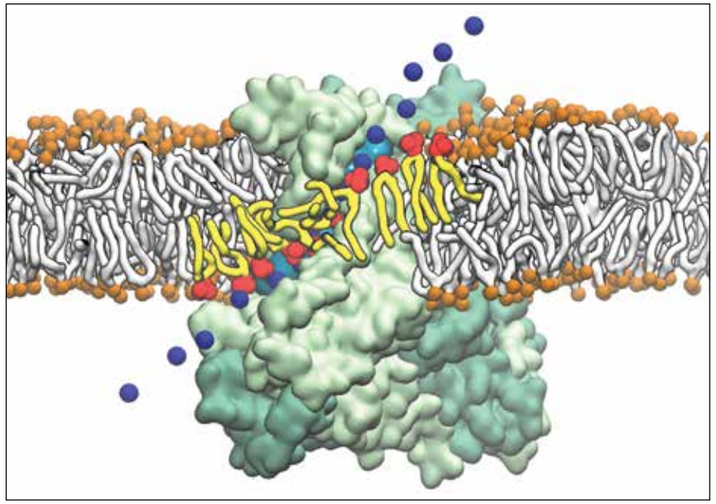

Figure 2: Lipids lining the hydrophilic track on the surface of the nhTMEM16 scramblase play a structural role in forming a "proteolipidic" pore, which is likely to be used by ions to cross the membrane. The permeating Na^+ ions during the simulations are shown in time series snapshots (blue spheres).

METHODS & CODES

We performed extended MD simulations on the atomic models of the nhTMEM16 scramblase in asymmetric lipid bilayers in the presence of Ca^{2+} ions at the activation binding sites. To uncover the nature of Ca^{2+} dependence, we also simulated a Ca^{2+}-free conformation of the protein in the same condition. To examine the ion permeation properties of the scramblase protein, we extended the fully equilibriated Ca^{2+}-activated simulation under multiple levels of applied transmembrane voltage, from which we calculated the ionic conductivity across the membrane. All MD simulations were carried out on Blue Waters using the NAMD (NAnoscale Molecular Dynamics) simulation package [7].

RESULTS & IMPACT

Our simulations reveal a significant deformation of the membrane structure induced by the nhTMEM16 scramblase protein due to its surface hydrophobicity (Fig. 1). The bending and thinning of the lipid bilayer primes lipid translocation by greatly reducing the energy barrier for hydrophilic head groups to move across the membrane. As the simulation was extended, a membrane-spanning lipid translocation track appeared on the protein surface (through hydration and occupancy of lipid head groups), effectively connecting the inner and outer leaflets of the bilayer (Fig. 1). In an aggregate 3 microseconds of Ca^{2+}-activated simulation, we observed one spontaneous full scrambling event through this track under equilibrium conditions and four full scrambling events in the presence of voltage. The observed track provides a hydrophilic environment for head groups to translocate between leaflets while the hydrophobic acyl chains are exposed to the hydrophobic phase of the bilayer. Simulations indicate that Ca^{2+} binding stabilizes the open conformation of the track by altering the structure of the lining transmembrane helices.

Our simulations also provide mechanistic insights into the ion channel properties of TMEM16 proteins, revealing that the membrane-spanning lipid track forms an ion-conducting "proteolipidic" pore between the protein and lipid head groups (Fig. 2). This flexible pore structure explains a number of unusual features of TMEM16 ionic currents, especially their highly variable ionic selectivity and ability to permeate large ions. In addition, key amino acids predicted by our simulations to enhance scrambling activity have been used successfully to experimentally engineer scramblase activity in a homologous Ca^{2+}-activated ion channel, thus providing insight into the evolutionary relationship of the TMEM16 family members.

WHY BLUE WATERS

The high-performance architecture of Blue Waters makes it an excellent computing resource for our scientific research. The GPU-accelerated simulation program NAMD has been extensively tested and optimized for Blue Waters. The large number of GPUs available in the XK nodes significantly increased our overall scientific productivity. Finally, the technical support provided by the expert scientists of the Blue Waters team has greatly facilitated the accomplishment of our research goals.

PUBLICATIONS & DATA SETS

Jiang, T., K. Yu, H.C. Hartzell, and E. Tajkhorshid, Lipids and ions traverse the membrane by the same physical pathway in the nhTMEM16 scramblase. *eLife*, 6:e28671 (2017), DOI:10.7554/eLife.28671.