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 (PIP2), 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 PIP2 binding sites in TMEM16A, a Cl− channel that performs key regulatory functions in the physiology. The research team has identified several PIP2 binding sites in TMEM16A, a Cl− channel that performs key regulatory functions in the physiology. These PIP2 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 PIP2–TMEM16A interactions provided by this research adds a crucial layer of information for understanding the regulation mechanisms of ion channels by specific lipids.

RESULTS & IMPACT
The research team developed atomistic molecular dynamics simulations performed on the atomic model of the ion channel using the highly mobile membrane mimetic model (HMMM) [12]. The HMMM 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. This model provides a more realistic 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 PIP2 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 PIP2 was influenced by full-length acyl chains, after the completion of lipid-binding simulations with HMMM membrane (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 NAnosecale Molecular Dynamics (NAMD) simulation package [13].

METHODS & CODES
To gain insight into the binding of PIP2 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 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. This model provides a more realistic 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 PIP2 molecules were added to the inner leaflet of an otherwise phosphatidylincholine (POPC) lipid bilayer evenly surrounding the protein at the beginning of the simulations. To determine whether binding of PIP2 was influenced by full-length acyl chains, after the completion of lipid-binding simulations with HMMM membrane (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 NAnosecale Molecular Dynamics (NAMD) simulation package [13].

RESULTS & IMPACT
The research team’s unbiased atomistic MD simulations with approximately 1.4% PIP2 in POPC bilayers revealed spontaneous binding of PIP2 to several potential sites on the surface of the TMEM16A channel (Fig. 1). Three of these sites captured 85% of all PIP2–protein interactions and were validated to be critical for PIP2 regulation through mutagenesis experiments by the collaborators. Simulations showed that PIP2 is stabilized by hydrophobic binding between basic residues and the phosphate/hydroxyl groups on the inositol ring of the lipid headgroup. Binding of PIP2 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 results in spontaneous penetration of Cl− ions into the pore (Fig. 2). Based on this observation, the research team proposed that a network of PIP2 binding sites at the cytosolic 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 Ca2+ and PIP2.

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 smoothly out technical issues that have arisen during the allocation.

PUBLICATIONS & DATA SETS