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The Importance of Membrane Defects—Lessons from Simulations

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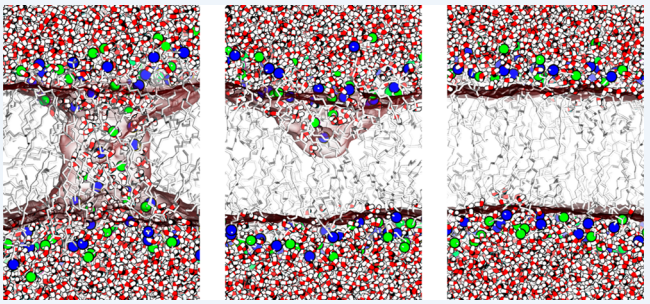
CONSPECTUS: The defects and pores within lipid membranes are scientifically interesting and have a number of biological applications. Although lipid bilayers are extremely thin hydrophobic barriers, just ∼3 nm thick, they include diverse chemistry and have complex structures. Bilayers are soft and dynamic, and as a result, they can bend and deform in response to different stimuli by means of structural changes in their component lipids. Though defects occur within these structures, their transience and small size have made it difficult to characterize them. However, with recent advances in computer power and computational modeling techniques, researchers can now use simulations as a powerful tool to probe the mechanism and energies of defect and pore formation in a number of situations.



In this Account, we present results from our detailed molecular dynamics computer simulations of hydrophilic pores and related defects in lipid bilayers at an atomistic level. Electroporation can be used to increase the permeability of cellular membranes, with potential therapeutic applications. Atomistic simulations of electroporation have illustrated the molecular details of this process, including the importance of water dipole interactions at the water−membrane interface. Characterization of the lipid−protein interactions provides an important tool for understanding transmembrane protein structure and thermodynamic stability. Atomistic simulations give a detailed picture of the free energies of model peptides and side chains in lipid membranes; the energetic cost of defect formation strongly influences the energies of interactions between lipids and polar and charged residues. Many antimicrobial peptides form hydrophilic pores in lipid membranes, killing bacteria or cancer cells. On the basis of simulation data, at least some of these peptides form defects and pores near the center of the bilayer, with a common disordered structure where hydrated headgroups form an approximately toroidal shape. The localization and trafficking of lipids supports general membrane structure and a number of important signaling cascades, such as those involving ceramide, diacylglycerol, and cholesterol. Atomistic simulations have determined the rates and free energies of lipid flip-flop. During the flip-flop of most phosphatidylcholine lipids, a hydrophilic pore forms when the headgroup moves near the center of the bilayer.

Simulations have provided novel insight into many features of defects and pores in lipid membranes. Simulation data from very different systems and models show how water penetration and defect formation can determine the free energies of many membrane processes. Bilayers can deform and allow transient defects and pores when exposed to a diverse range of stimuli. Future work will explore many aspects of membrane defects with increased resolution and scope, including the study of more complex lipid mixtures, membrane domains, and large-scale membrane remodeling. Such studies will examine processes including vesicle budding and fusion, non-bilayer lipid phases, and interactions between lipid bilayers and other biomolecules. Simulations provide information that complements experimental studies, allowing microscopic insight into experimental observations and suggesting novel hypotheses and experiments. These studies should enable a deeper understanding of the role of lipid bilayers in cellular biology and support the development of future lipid-based biotechnology.

INTRODUCTION

Biological membranes are soft structures that bend and deform easily. At the same time, however, they are complex structures that adapt to varying conditions. Transient pores form when they are stretched or placed in an electric field, during translocation of cationic peptides, in intermediates in vesicle fusion, and during ion transport and lipid flip-flop. Smaller lipid deformations are important in membrane function: for instance, hydrophobic mismatch between the lipids and protein can induce lipid deformation and peptide tilt and affect the properties of ion channels.[1]

The structural integrity of cellular membranes is paramount for the proper function of cells and organelles. Specific molecules must be allowed to cross the membrane at specific times, such as metabolites or ions during nerve cell propagation. Others molecules must be barred, creating concentration gradients essential as a source of energy. Specialized channels and transporters create and maintain the necessary concentration gradients across membranes, but passive diffusion across membranes also occurs. The molecular

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composition of a membrane directly affects its permeability and structural integrity. Cells and cellular organelles have different and varied lipid compositions, with thousands of unique lipid types.[2] Defining the physicochemical properties of bilayer defects may allow advances in drug delivery and novel therapeutics and improve our understanding of membrane protein structure and function.

Computer simulations of lipid membranes have allowed us to explore many problems related to pore and defect formation. In this Account, we present results on the mechanism and energetics of lipid membrane defect formation provided by atomistic molecular dynamics simulations, with an emphasis on our own work. While there are more comprehensive reviews that include sections on membrane defects,[3,4] we have attempted to bridge the underlying mechanisms of a number of different membrane phenomena that involve defects.

■ COMPUTER SIMULATIONS OF MEMBRANES

Atomistic simulations of lipid membranes became feasible in the early 1990s (as reviewed in refs 5 and 6). As a result of limited computational power, early simulations primarily focused on the equilibrium structure and dynamics of small patches of membrane on time scales up to a few nanoseconds. Coarse-grained modeling has proven extremely useful for studying large-scale lipid and surfactant systems.[7−10] One to two decades later, computers are orders of magnitude more powerful, allowing more elaborate and detailed simulations of biologically relevant processes. Perturbed and deformed membranes were first simulated around a decade ago, with papers on perturbed bilayers as part of the self-assembly of bilayers and vesicles,[11,12] electroporation,[13] and mechanical stress[13−16] and with early coarse-grained models.[17]

The crystal structure of KvAP[18] raised the puzzling possibility of having multiple lipid-exposed arginine residues and focused the field toward arginine−lipid interactions and water defect formation. Early simulations of the voltage sensor "paddle" in lipid membranes showed significant defects, which hinted at non-Born behavior as a key determinant for partitioning.[19−21] Because of the complexity and sampling difficulties inherent in simulations of large membrane protein systems, many simulation studies focused on side chains and small model peptides. Concurrently, there were simulations on pore formation with antimicrobial peptides[22] and electroporation.[13] Applying a lateral tension to a bilayer was shown to induce pore formation.[13] These early studies of membrane pores and defects have spurred numerous research directions that have characterized lipid bilayer deformations with great detail. Here we focus primarily on simulations in atomistic detail, as the small length scale of the phenomena of interest puts extreme demands on coarser models.

■ ELECTROPORATION

Applying an electrochemical potential across lipid bilayers can cause membrane disruption. This is routinely used in biotechnology, for example in transfection of DNA into cells. The goal is to tune the applied potential to selectively kill diseased or microbial cells as well as to enhance the uptake of charged or polar drugs into specific cells. Experiments can yield macroscopic parameters related to pore formation, but understanding the molecular-level details is difficult.

Early on, we showed that applying a constant electric field across a dipalmitoylphosphatidylcholine (DPPC) bilayer caused a hydrophilic pore to form.[13,14] Figure 1 illustrates the standard view of lipid bilayer electroporation from simulations. Initially, a

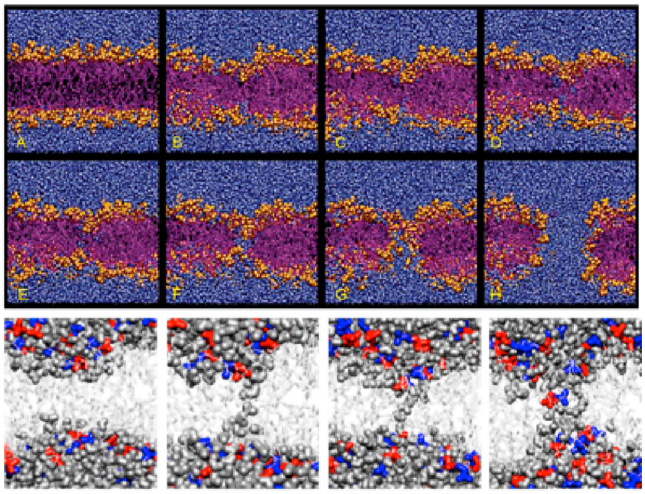

Figure 1. Mechanism of pore formation. (A−H) The process of electroporation in a DPPC bilayer. Waters are shown as blue balls; DPPC headgroups and tails are colored orange and magenta, respectively. (Reprinted from ref 13. Copyright 2003 American Chemical Society.) Bottom panels: Pore formation in a DLPC bilayer during an equilibrium simulation.[31] Water is shown in silver; DLPC headgroups are colored red and blue, and the tails are colored gray. (Reprinted with permission from ref 31. Copyright 2014 the authors of ref 31.)

small water wire is observed, followed quickly by the diffusion of lipid headgroups into the bilayer. The pore widens, with multiple headgroups and water forming a toroidal shape. The interaction of the external field and the resulting strong local field gradients at the water−lipid interface with the dipoles of individual water molecules was shown to be the key driving force for poration.[14,23,24] Lipid headgroup dipole rearrangements do not have as large of a role, especially given that similar pores are observed in an octane slab[14] and a vacuum slab system.[23,25] Recent coarse-grained models that have a dipole for water are able to capture membrane electroporation,[26,27] which further supports the importance of water's dipole in electroporation. However, headgroup rearrangements are clearly necessary as lipids enter the membrane interior to form a toroidal pore. Perturbed headgroup tilt was shown to be an important prepore state in the overall mechanism.[24,28] A systematic study of membrane electroporation and a statistical model relating the microscopic, single pore event kinetics from simulations to macroscopically observed experimental kinetics showed good agreement between simulations and experiments.[28] The creation of an ion imbalance across a lipid bilayer has also been used to induce pore formation in simulations with a very similar mechanism.[29,30] We recently observed spontaneous pore formation in a thin dilauroylphosphatidylcholine (DLPC) bilayer under equilibrium conditions[31] and found a similar mechanism as for electroporation, with a short-lived water wire spanning the bilayer (Figure 1). The similarity in the mechanism and structure of pores in unperturbed lipid bilayers during electroporation in octane and vacuum slabs suggests common underlying molecular driving forces.

Understanding of the molecular mechanism for pore resealing is needed to minimize leakage from nontargeted cells and for the delivery of charged molecules into the cell. The

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mechanism of pore closure from simulations is the opposite process as opening, with the headgroups exiting the pore first, leaving a short-lived water wire across the membrane, which then closes quickly.[16,32] Pore lifetimes are on the order of nanoseconds to hundreds of nanoseconds and depend critically on the structure of the bilayer.[29,31−33]

SIDE-CHAIN MEMBRANE DISTRIBUTIONS

Understanding membrane protein folding, stability, and function is a key problem in biology. Hydrophobicity scales have been used widely and relatively successfully to predict a transmembrane protein's topology from its sequence. In addition to a large number of scales based on statistical properties, there have been a number of experimentally derived hydrophobicity scales for the 20 amino acids. The first scales were based on the partitioning of small-molecule side-chain analogues between bulk solvents,[34] followed by scales based on partitioning of small-model peptides between bulk solvents and interfaces.[35] More recently, experiment-based hydrophobicity scales have been determined using the reversible insertion of a mutated peptide using the Sec translocon machinery[36] and the folding and insertion of the mutated OmpLA β-barrel protein.[37] Understanding the molecular details for each experiment will likely explain the discrepancies between the different scales.[38] For example, simulations showed that the OmpLA protein is stable in a DLPC bilayer, even with a mutated arginine at the bilayer center, as a result of the formation of a water defect to keep it solvated.[39]

Free energy profiles for the small-molecule analogues of the amino acid side chains moving from water into a dioleoylphosphatidylcholine (DOPC) bilayer were calculated by atomistic simulations.[40−42] As expected, the hydrophobic side chains had the lowest free energy near the center of the bilayer. Tryptophan had a strong preference for the interface, which supports its well-known role in stabilizing transmembrane peptides and proteins.[43] However, polar and charged amino acids showed surprisingly complex behavior. Water defects were observed as they entered the bilayer's hydrophobic interior. For the polar side chains, there was an energetic balance where at a certain distance from the bilayer center the cost of forming a defect was greater than the cost of desolvating the side chain. The charged side chains remained solvated throughout the bilayer, even at the center, causing a significant defect in the membrane (Figure 2). The free energy scale for all of the amino acids from our MD simulations compared well to the experimental scales, which also compared reasonably well to one another in their relative values but not in their respective magnitudes.[38]

Arginine has been the most studied amino acid in this context because of its importance in ion channels, antimicrobial peptides, and cell-penetrating peptides. Atomistic simulations have determined the free energy of transfer from water to the center of a lipid bilayer for arginine on a polyleucine helix,[44] an OmpLA β-barrel protein,[45] and its small-molecule analogue propylguanidinium.[40,41,46] The free energy of transfer from the MD simulations was much lower than expected from a simple continuum Born-like model.[44] This is due to deformation of the lipid bilayer to allow the water to hydrate the guanidinium group even at the center of a DOPC bilayer (Figure 3). While this behavior was reproduced with more accurate polarizable models,[47,48] coarse-grained models have failed to capture the defect formation.[47,49] Many simulations have shown that arginine can remain protonated and positively charged even

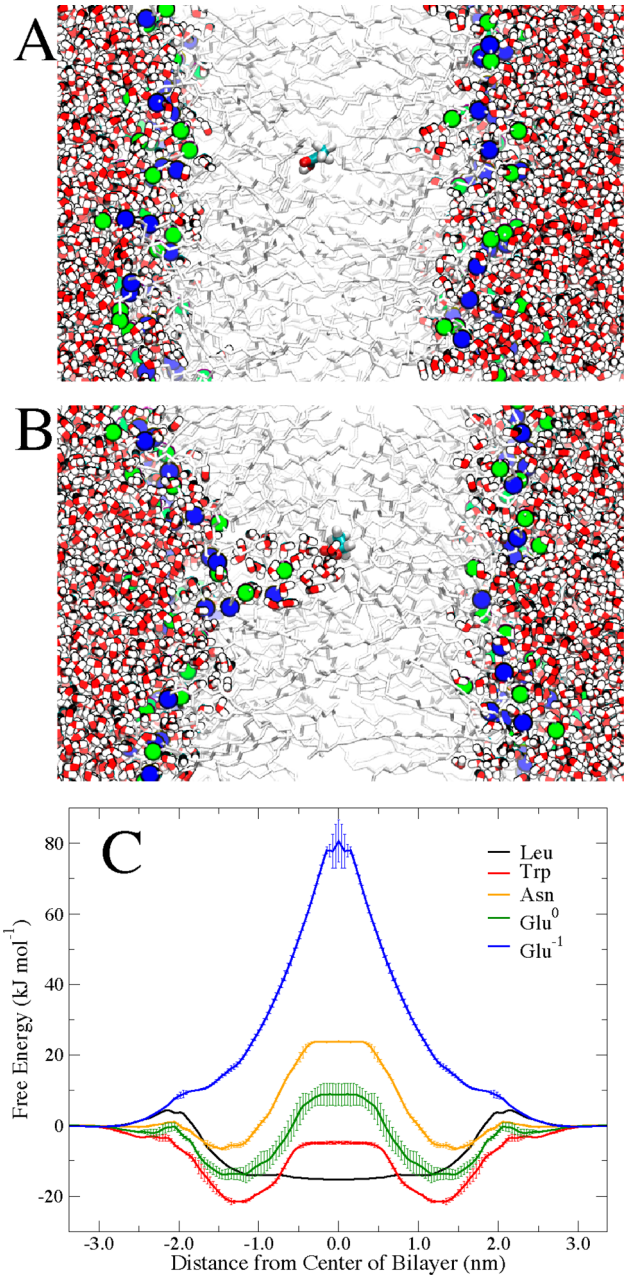

Figure 2. Amino acid side-chain free energies in a DOPC bilayer. (A) Neutral and (B) negatively charged glutamic acid restrained at the center of a DOPC bilayer. Waters are shown as red and white lines, DOPC tails as gray lines, nitrogen atoms as green balls, phosphorus atoms as blue balls, and the side chains as thick licorice. (C) Free energy profiles for a select set of amino acid side chains from ref 41.

at the center of the bilayer.[41,44,46] This is in contrast to the other charged side chains, which would likely (de)protonate within the bilayer core.[41] Experiments recently confirmed that arginine but not lysine remains charged in the center of the membrane.[50] The bilayer composition and thickness were shown to have a large effect on the free energy and diffusion mechanism for arginine permeation, with small pores formed in thin lipid bilayers.[51]

We determined the free energy to transfer a second arginine into a DOPC with an initial arginine restrained at the bilayer center (Figure 3).[52] The free energy was much lower than for the first one. The high energetic cost for the first arginine is due

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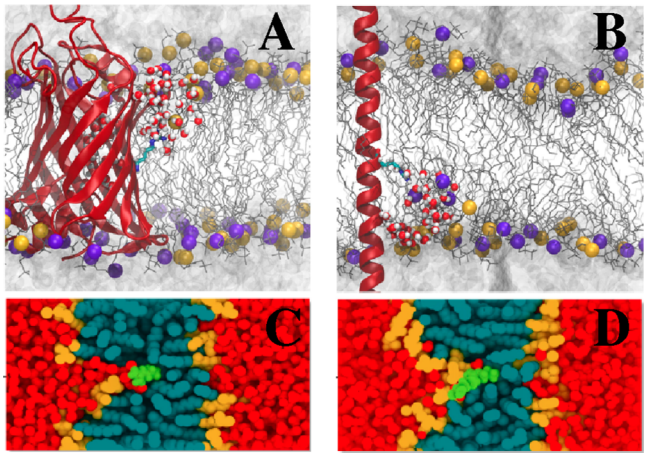

Figure 3. Arginine in lipid bilayers. (A, B) Arginine on an OmpLA protein (A) and a polyleucine model peptide (B) in a DLPC bilayer. (Reprinted with permission from ref 45. Copyright 2012 The Biophysical Society.) (C, D) A single arginine restrained at the center of a DOPC bilayer (C) and two arginine side chains at the center of the bilayer (D). (Reprinted with permission from ref 52. Copyright 2011 The Biophysical Society.)

to the deformation of the bilayer, which the second arginine does not have to "pay". This concept was shown experimentally using the Moon and Fleming hydrophobicity scale based on the folding and insertion of OmpLA, with a second arginine near the first in the mutated structure.[37] The fact that bilayer deformation is the major energetic barrier implies that the collective properties of the membrane are crucial for the transfer of charged molecules across lipid bilayers. For integral membrane protein structure and stability, this means that the protein's structure and activity depend on the bilayer's properties.[1] This is also important for targeting and transport strategies: each arginine of a polybasic antimicrobial or cell-penetrating peptide can cross in the same single bilayer pore.

Free energy calculations for small molecules in lipid bilayers are also useful for method development. Our side-chain free energy profiles have been used to help parametrize coarse-grained force fields[49,53] and implicit membrane models.[54] Because of the complex nature of the process of solute partitioning across lipid bilayers and the extensive number of simulations, these types of calculations can be used for critical sampling tests[55] and improved sampling methodology.[56]

■ ANTIMICROBIAL AND CELL-PENETRATING PEPTIDES

There are a large number of small and charged peptides that are able to form hydrophilic pores in lipid bilayers. This is one of the commonly assumed mechanisms for antimicrobial peptide (AMP) activity,[57] where the usually cationic peptides porate bacterial membranes but not eukaryotic membranes. Some peptides are also effective against cancer cells, likely as a result of the negatively charged lipids exposed on cancer cell membranes,[58] which is similar to the situation for bacterial cells. Cell-penetrating peptides (CPPs) are short polycationic peptides that are able to translocate cell membranes and deliver relatively large cargo molecules, with applications for drug and small interfering RNA (siRNA) delivery.

There have been a large number of simulations on the interactions of AMPs and CPPs in lipid bilayers (as reviewed in refs 3 and 22). Several simulations have shown that a number of AMPs and CPPs form pores in model membranes that have structures very similar to those observed during electroporation, with a disordered toroidal structure[59−61] (Figure 4).

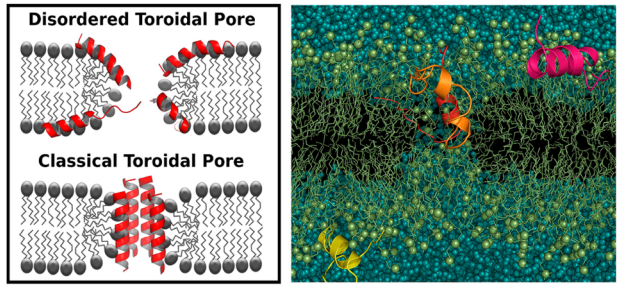

Figure 4. (left) Schematic comparing the structures of disordered and "classical" toroidal pores in a lipid bilayer; (right) atomistic simulation of melittin peptides in a DPPC bilayer with a hydrophilic pore. (Reprinted with permission from ref 60. Copyright 2008 Elsevier B.V.)

The free energy profile for the CPP penetratin showed a barrier of ∼75 kJ/mol for translocation across a DPPC bilayer, suggesting a possible but rather expensive mechanism of pore formation for internalization of CPPs.[62] In one study, the free energy barrier for transfer of a cyclic nonaarginine peptide from water to the center of a DOPC bilayer was lowered by 80 kJ/mol when a pore was already present, although it still had a significant barrier of ∼120 kJ/mol.[63]

There have been a number of studies of pore-forming peptides using the MARTINI coarse-grained (CG) model, although the ability of MARTINI to accurately describe this process is limited.[7] In a number of interesting cases, CG simulations have been used initially to observe peptide aggregation, and then the system is switched to atomistic resolution to observe the finer details of pore formation.[64,65]

In contrast to charged peptides, even quite large hydrophobic molecules do not appear to cause enough perturbation to porate membranes. For instance, although membrane disruption was one proposed mechanism for fullerene−lipid interactions, simulations showed no defects,[66] and neither did nanotubes of various types.[67]

■ LIPID FLIP-FLOP

Lipid flip-flop is an important cellular process for membrane growth and signaling processes, for example the exposure of phosphatidylserine in apoptosis.[68] Phospholipid flip-flop is expected to be a relatively slow process because of the large and zwitterionic or charged headgroups of the lipids, with measured time scales on the order of hours or more for PC lipids. We used umbrella sampling calculations to determine the free energy profile for moving a single lipid headgroup from equilibrium into the center of the lipid bilayer, which we assumed was the free energy barrier for flip-flop.[69] For DPPC, we found that when the phosphate was near the center of the bilayer, a pore formed at a free energy cost of ca. 80 kJ/mol.[69] The free energy for pore formation agreed with a previous calculation using the pore radius as the reaction coordinate.[70] From the free energy barrier, the pore lifetime, and the flux of lipids across the pore, we estimated the rate of lipid flip-flop to be on the time scale of hours, in good agreement with experimental estimates.[69] As the headgroup entered the hydrophobic region, a water defect formed, similar to the one for arginine partitioning, with nearly the same slope in the free

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energy profile (Figure 5). We found that pores form orders of magnitude more frequently in thinner DLPC bilayers compared

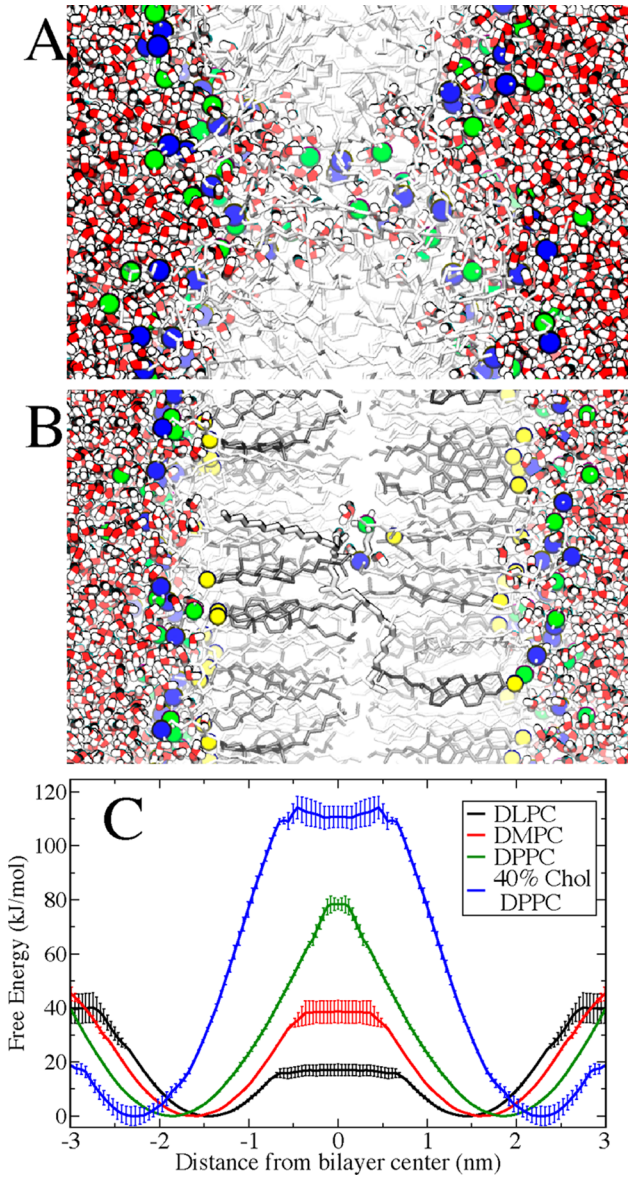

Figure 5. Lipid flip-flop in model membranes. (A) The phosphate of a single DMPC restrained at the center of a DMPC bilayer. (B) The phosphate of a single DPPC restrained at the center of a DPPC bilayer with 40% cholesterol. The representations are the same as in Figure 2. (C) Free energy profiles for lipid flip-flop in a number of model membranes from refs 69, 71, and 72.

with DMPC and DPPC.[31,71] Increasing the cholesterol content in a DPPC bilayer further increased the free energy barrier for DPPC flip-flop and prevented pore formation.[72] We found that the free energy cost for DMPC pore formation was due to a large unfavorable entropic contribution and favorable enthalpy, likely because of increased water–lipid headgroup interactions.[31]

The localization of cholesterol and other signaling lipids is of particular interest given their role in health and disease. Using atomistic and coarse-grained MD simulations, we determined the free energy profiles and the rate of cholesterol flip-flop across a number of diverse model membranes.[73,74] Similar free energy barriers for cholesterol flip-flop were found using a different force field.[75] In contrast to phospholipid flip-flop, we found that cholesterol translocates by a solubility–diffusion mechanism in desolvated form without the formation of a pore or water defect at the center of the membrane. We found high rates of flip-flop across liquid-disordered membranes and orders of magnitude lower rates in liquid-ordered bilayers with high cholesterol content. The results for the atomistic and coarse-grained models were in good agreement for the most part, but the coarse-grained simulations allowed us to directly observe flip-flop in long equilibrium simulations. Several signaling lipids have uncharged, hydrophilic headgroups that are sufficiently small that they might flip-flop rapidly on biological time scales. For ceramide and diacylglycerol, we calculated translocation rates across liquid-disordered and liquid-ordered bilayers, finding orders of magnitude faster flip-flop across the disordered membrane.[73] Matching experimental predictions, we found that the rates of flip-flop increased in the order diacylglycerol < ceramide ≪ PC.[76] This was in qualitative agreement with CG simulations of ceramide flip-flop.[77]

For lipid flip-flop and transient pore formation, we compared atomistic and CG free energies for DPPC and DLPC (Figure 6).[78] In the MARTINI model, we did not observe pore

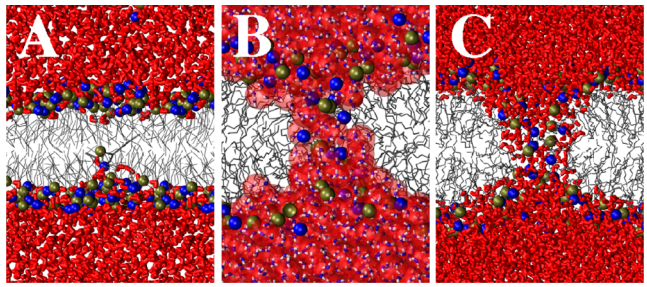

Figure 6. Pores in short lipid bilayers. (A) Coarse-grained simulation with the phosphate of a single DLPC lipid at the center of a DLPC bilayer with no pore in the bilayer. (B, C) Pores in atomistic DMPC bilayers with the phosphate of a single DMPC restrained at the bilayer center. In (B), four waters were tethered together to test the effect of 4:1 mapping used for MARTINI water. Waters are shown in red, lipid tails as gray lines, phosphates as tan balls, and cholines as blue balls. (Reprinted from ref 78. Copyright 2011 American Chemical Society.)

formation for the thin DLPC bilayer or the thicker DPPC bilayer, as expected from atomistic simulations. The barrier for DLPC was much higher with the MARTINI water (80 kJ/mol) than with the atomistic model (18 kJ/mol).[78] On the basis of the atomistic free energy decomposition for pore formation discussed above, it is understandably difficult for a CG model that has reduced entropy to reproduce pore formation accurately.

■ WHAT LED TO PROGRESS?

As the field of lipid bilayer simulations has progressed, the realization that many diverse processes have similar molecular mechanisms has advanced the field. Many commonalities have been observed in the simulations discussed above, and perhaps more can be learned from the general themes than from the specific and diverse results. For example, spontaneous pore formation in a thin DLPC bilayer[31] showed a similar mechanism as electroporation. Water is emerging as a key player in bilayer deformations, which is not surprising given the energetic cost of desolvating a charged solute as well as the importance of water entropy in the hydrophobic effect. It is

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energetically more favorable for the bilayer to deform than for a charged molecule to enter the hydrophobic bilayer interior. By bending, the bilayer−water interface changes shape, and the interactions between water and the lipid are modified. The collective interactions and energetics for bilayer defect formation are another theme that has emerged from simulations. Once a pore or defect is formed, the energetic barrier for charged or polar molecules to cross the membrane is significantly reduced. The importance of collective interactions is illustrated by the large effect of the composition and structure of the membrane on the energetics of pore and defect formation.

The constant increase in computer power has enabled atomistic simulations of larger systems for longer time scales. What is routinely published today would not have been possible five or ten years ago. Because of the slow and correlated motions of lipids, hundreds of nanoseconds is needed to sample even relatively fast processes in membranes, and much longer times are required for processes such as flip-flop. Improved computational techniques, sampling methods, and coarse-grained model development has helped speed progress. Modeling of water with CG models is difficult because of the small size of individual molecules, which may explain why CG models do not reproduce pore formation accurately.

■ OUTLOOK

Defining the physicochemical properties of bilayer defects may allow advances in drug delivery and novel therapeutics and improve our understanding of membrane protein structure and function. While we have learned a great deal about the molecular mechanism and energetics of pores using MD simulations, there are many avenues to pursue for investigating membrane defects and pores. Sampling remains a concern for many atomistic simulations given the long-time-scale processes in lipid systems. Large-scale processes, including formation of pores in membrane domains, the interaction with large membrane proteins, and vesicle fusion and budding, will be simulated with increasing resolution. Quantitative studies and free energy calculations of antimicrobial peptide and cell-penetrating peptide interactions with lipid membranes will be conducted with increased sampling and more realistic systems. The effect of pH on many different processes involving membrane defects will be possible in the near future.[79,80] More detailed CG and continuum models will be developed, as well as multiscale methods linking the different models, leading to truly macroscopic simulations. Simulations and experiments will become more integrated, allowing direct theoretical validation and experimental interpretation, novel hypotheses, and a more complete picture of membranes. Simulations have provided fundamental insight into membrane defects, and the prospects for future discoveries is extremely promising.

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Notes

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