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Solvent-exposed lipid tail protrusions depend on lipid membrane composition and curvature

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ABSTRACT

The stochastic protrusion of hydrophobic lipid tails into solution, a subclass of hydrophobic membrane defects, has recently been shown to be a critical step in a number of biological processes like membrane fusion. Understanding the factors that govern the appearance of lipid tail protrusions is critical for identifying membrane features that affect the rate of fusion or other processes that depend on contact with solvent-exposed lipid tails. In this work, we utilize atomistic molecular dynamics simulations to characterize the likelihood of tail protrusions in phosphotidylcholine lipid bilayers of varying composition, curvature, and hydration. We distinguish two protrusion modes corresponding to atoms near the end of the lipid tail or near the glycerol group. Through potential of mean force calculations, we demonstrate that the thermodynamic cost for inducing a protrusion depends on tail saturation but is insensitive to other bilayer structural properties or hydration above a threshold value. Similarly, highly curved vesicles or micelles increase both the overall frequency of lipid tail protrusions as well as the preference for splay protrusions, both of which play an important role in driving membrane fusion. In multicomponent bilayers, however, the incidence of protrusion events does not clearly depend on the mismatch between tail length or tail saturation of the constituent lipids. Together, these results provide significant physical insight into how system components might affect the appearance of protrusions in biological membranes, and help explain the roles of composition or curvature-modifying proteins in membrane fusion.

1. Introduction

Phospholipids are biological amphiphiles consisting of two hydrophobic tails and a hydrophilic head group that self-assemble into a characteristic bilayer structure in water. The lipid bilayer is one of the primary components of the cell membrane and is critical for regulating transport into and out of the cell. For example, the low dielectric constant of the hydrophobic tail region minimizes the passive diffusion of water, ions, and other small hydrophilic molecules through the membrane [1]. As a result, water-soluble molecules are often encapsulated within lipid vesicles to facilitate intra- and extracellular trafficking. The exchange of molecules between such vesicles, or between vesicles and other lipid-bound compartments, occurs after membrane fusion. In this process, two lipid bilayers merge to form a single connected structure that allows the mixing of contents from the previously distinct compartments. Fusion between lipid vesicles is one of the primary mechanisms for the transport of hydrophilic small molecules, lipids, or proteins throughout the cell; for example, endosomes containing internalized material will fuse with endosomal sorting complexes as part of a recycling pathway [2]. Similarly, fusion between internal vesicles and the outer membrane triggers the secretion of soluble proteins to the extracellular environment during exocytosis and is necessary for the transport of neurotransmitters at synapses [3,4]. These biological fusion processes are generally accelerated by membrane-bound SNARE proteins from opposing membranes forming a supramolecular complex that facilitates membrane proximity and enables lipid mixing to drive stalk formation and membrane fusion[5,6].

Given the biological relevance of membrane fusion, there has been significant work aimed at understanding its physical mechanism. The generally accepted fusion pathway involves three intermediate states with corresponding energy barriers [7–9]. First, two bilayers must approach within a distance of approximately 0.9 nm [10], which requires overcoming a repulsive hydration force associated with dehydrating the intervening solvent region, [10–12]. Next, contact between hydrophobic lipid tails in the two apposed bilayers nucleates the formation of a lipid stalk, or a highly curved lipid bridge formed from the mixture of the two proximal lipid monolayers [13,14]. The stalk then radially expands into a hemifusion intermediate to reduce the bending energy of the disrupted lipids [7,15]. Finally, full fusion occurs if the hemifusion intermediate ruptures and forms an aqueous channel through the two bilayers that allows for the transfer of contents [16,17]. Experimental approaches have been valuable in characterizing several of these transitions, including the magnitude of the repulsive hydration force [10,11], barrier to lipid mixing [15], and expansion of the stalk [16,18]. However,

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detailed molecular insight into the nature of these transitions is difficult to assay experimentally but is necessary to understand the role of various components in the fusion pathway, such as fusion proteins [19] or different lipid compositions [10,20].

Recently, computer simulations have become increasingly valuable in understanding the mechanism of fusion by providing this missing molecular detail [9,21–30]. In particular, particle-based molecular simulations are capable of resolving the lipid mixing that marks the onset of stalk formation. Multiple different simulation methodologies have led to the identification of a pre-stalk transition involving the stochastic protrusion of hydrophobic lipid tails into the solvent layer between two vesicles [23,31–35]. Contact between protruding lipid tails has been established as a transition state prior to stalk formation; if such contact occurs, the pre-stalk intermediate may relax to a metastable stalk [32,33,36]. As a result, it is critical to understand the conditions under which tail protrusions are promoted in order to identify membrane components that encourage the pre-stalk transition. Moreover, similar lipid tail protrusions have been shown to trigger the spontaneous insertion of amphiphilic nanoparticles into lipid bilayers [37], raising the possibility that these stochastic fluctuations may be important in other processes involving contact between hydrophobic molecules and the bilayer core. Molecular dynamics simulations and neutron diffraction experiments have provided direct evidence for the incidence of these protrusions, with a substantial concentration of acyl-chain methyl groups observed to intercalate with lipid head groups and access interfacial solvent molecules in phospholipid bilayers [38]. In addition, recent simulations have examined related lipid fluctuations, such as the formation of hydrophobic defects [39–41] or lipid desorption [42,43]. To the best of our knowledge, however, no substantial study has yet been performed to systematically investigate the factors that influence the likelihood of lipid tail protrusions in protein-free bilayers.

In this work, we use atomistic molecular dynamics simulations to quantify the likelihood of observing the spontaneous protrusion of a single lipid tail into aqueous solution. We further use umbrella sampling simulations to calculate the potential of mean force (PMF) required to induce a protrusion, then compare PMFs for several different lipid compositions. The PMFs show that the free energy cost for a protrusion depends on lipid tail saturation and the location of the protruding lipid tail atom but is insensitive to tail length in lipids with phosphatidylcholine head groups. The preference of atoms near the head group or near the tail end to protrude leads to the classification of both “elbow” and “splay” protrusion modes, respectively. Moreover, we find a significant increase in the PMF below a critical level of bilayer hydration corresponding to the interpenetration of lipid head groups. To supplement these free energy calculations, we calculate the protrusion likelihood from unbiased simulations of bilayers with different compositions, curvature, and hydration. We find that protrusions occur on an approximately 100 ns timescale depending on the extent of solvent exposure. Crucially, we observe that bilayer curvature can substantially increase the incidence of these protrusion events relative to planar bilayers. This work shines significant physical insight into the likelihood of observing spontaneous protrusions in planar and curved homogeneous bilayers and how membrane features like composition, curvature, and hydration can affect protrusion propensity, which has applications in understanding and potentially manipulating this barrier in processes of biological interest.

2. Methods

Atomistic molecular dynamics simulations were used to model a series of lipid bilayers to quantify protrusion likelihood. Five different single-component lipid bilayers—containing DLPC, DMPC, DPPC, POPC, or DOPC—were constructed by extracting 64 lipids from the 128 lipid pre-equilibrated bilayers provided by Poger et al. [44]. All five lipid species have zwitterionic phosphatidylcholine head groups but differ in the number and saturation of carbon atoms in the tail groups (chemical

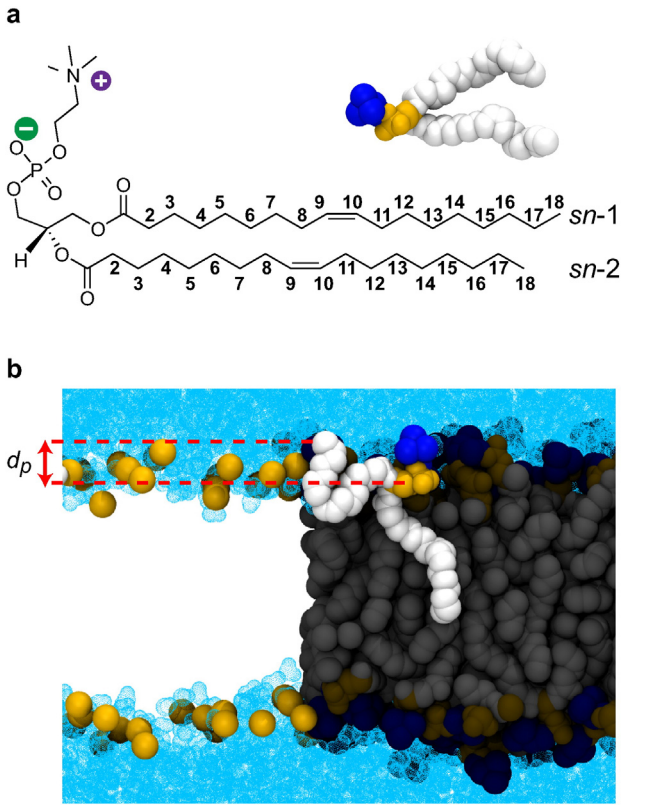

Fig. 1. Lipid structure and protrusion definition. **a** Chemical structure of DOPC and simulation representation. Atom indices in both tails are numbered starting from the glycerol group. **b** Example protrusion and definition of d_p, the distance between a hydrophobic tail atom and the same lipid's phosphorus atom projected along the membrane normal. Several additional phosphorus atoms are shown to illustrate that they are roughly coplanar in the bilayer. Lipid tails are in white, the phosphate group in yellow, the choline group in blue, and water in cyan. All images were generated using Visual Molecular Dynamics [52].

structures shown in Fig. 1 and Fig. S1). The 64 lipids were resolvated to have 50–60 water molecules per lipid. One bilayer, composed of DOPC, was simulated at both 300 K and 323 K. All other bilayers were run at 323 K, a temperature well above the gel-fluid phase transition (Table S1). We also investigated the effect of bilayer hydration, curvature, and inhomogeneity on the likelihood of lipid tail protrusion. 64 lipid DOPC bilayers were prepared with 5, 7, 10, 25, and 60 water molecules per lipid to vary the water layer thickness and simulated at 300 K. Bilayers constructed with a binary mixture of lipids that differed in either tail length (DPPC/DLPC) or tail saturation (DPPC/DOPC) were prepared through a reverse coarse-graining procedure [45] using the MARTINI force field [46] as described in the Supplementary Information (Fig. S2). Each mixed bilayer contained 512 lipids in a 1:1 ratio of the two components, with the larger bilayer size permitting suitable lipid interdiffusion, and was simulated at 323 K. Finally, a 50 lipid DOPC micelle and a 900 lipid DOPC vesicle were both constructed using a similar reverse coarse-graining procedure (Fig. S2). Physical properties of all systems simulated are summarized in Table S1.

All systems were modeled using the GROMOS 54a7 force field with the SPC water model [44,47,48]. A leap-frog molecular dynamics integrator was used with a timestep of 2 fs. System configurations were saved every 20 ps. All bonds were constrained using the LINCS algorithm [49]. Long-range electrostatic interactions were modeled using the smooth particle mesh Ewald (PME) method with a grid spacing of 0.12 nm and fourth-order interpolation. The neighbor list cutoff, van der Waals cutoff, and short-range electrostatics cutoff were all set to 1.0 nm to match recent simulation methods [50]. Constant temperature was maintained at either 300 K or 323 K using a velocity-rescale thermostat with a timestep of 0.1 ps. Constant pressure was maintained at