Platform: Membrane Physical Chemistry II

909-Plat
Can Lipids be used as Mobility Standards in Artificial Bilayers?
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The literature data and results presented in this article show that in lipidic bilayers lipids diffuse with various diffusion coefficients ranging from D = 3.7 ± 0.4 to 13.9 ± 0.6 μm2s-1, Interestingly, a transmembrane peptide, having nearly the same radius as lipids and whose hydrophobic thickness matches that of the bilayer, exhibits a D value of 9.6 ± 0.4 μm2s-1. Since the lipids and the transmembrane peptide possess a similar diameter and lipids do not span the whole membrane, the diffusion coefficient of peptide is expected to be smaller than that of lipids, which is not the case in our experiments. Our systematic study and reinterpretation of literature data suggests that the slower diffusion of lipids, as compared to that of the transmembrane peptide, is caused by the formation of dynamic lipid nanopatches that diffuse like a single object with an increased radius. These nanopatches form more spontaneously when lipids are saturated. Molecular dynamics simulations confirm our experimental results and show that the nanopatches formation also occurs in bilayers made of single lipid in absence of dye.
Consequently, one should be cautious when comparing the mobility of transmembrane proteins and lipids. Transmembrane peptides should be used as a reference instead of lipids.

910-Plat
Cu^{2+}-Phosphatidylserine Binding and its Implications for Protein-Membrane Interactions
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Phosphatidylserine (PS) lipids in cell membranes play a significant role in apoptosis, blood clotting and neurodegenerative disease such as Alzheimer's disease (AD). Using a novel fluorescence quenching assay, PS lipids in supported lipid bilayers (SLBs) were found to bind Cu^{2+} with high affinity. The resulting Cu^{2+}-PS complex can quench a broad spectrum of lipid-bound fluorophores in a reversible and pH-dependent fashion. This quenching assay can be used to determine the binding affinity between Cu^{2+} and SLBs containing various PS concentrations, since the PS concentration on the cell surface changes during different biological events. It can be also used to investigate Cu^{2+}-mediated interactions between proteins and membranes, for example, to explore β-Amyloid peptide (Aβ)-membrane interactions to assist clarifying the membrane-related neurotoxicity of Aβ.

911-Plat
Protein-Free Membrane Fusion Probed by Single Giant Unilamellar Vesicle Imaging - the Role of Membrane Charge
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Membrane fusion is a ubiquitous process in biology. The outcome of fusion is followed by successive steps of fusion intermediates, including (i) protein tethering in apposing bilayers, (ii) membrane adhesion, (ii) hemifusion and eventually (iv) full fusion, the latter leading to complete mixing of membranes and aqueous contents. Studying membrane fusion in vivo is, however, very challenging. By a combination of microscopic approaches, we investigate the role of membrane charges on the interaction and fusion of cationic large (DOTAP:DOPE:DPPE-Rh - 1:1:0.1 mol) and individual giant unilamellar vesicles (LUVs-GUVs) in a pure lipid system. Fluorescencence Resonance Energy Transfer (FRET) efficiency, in which the donor is reconstituted in the GUVs and the acceptor incorporated upon membrane merging, is measured and directly related to fusion efficiency. LUVs stably adhere and diffuse onto the surface of neutral (POPC) GUVs with minimal membrane merging (FRET efficiency 0.1). In sharp contrast, very efficient fusion occurs with the negative GUV membranes (POPC:POPG - 1:1) - FRET 0.6-1. An increase in membrane area after contact with the fusogenic LUVs was clearly detected for this composition. Excess of negative lipids on GUVs (pure POPG) relative to positive charges decrease fusion efficiency (FRET 0.3) - a charge-neutralization process. Transferred lipids upon fusion undergo similar diffusion to those on GUV membranes (~ 1 μm2/s) With the developed LUV-GUV system, we studied the charge-dependent membrane adhesion and fusion separately and individually for the very same interacting partners, not possible with traditional in vitro systems. The system could also be easily adapted to protein-reconstituted studies.
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912-Plat
Helix Insertion Drives Membrane Bending by Enabling Protein Crowding
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A key physical chemistry problem in cell biology is the generation of highly curved membrane structures. The epsin1 N-terminal homology (ENTH) domain is a well-studied curvature-generating protein widely believed to shape endocytic pits by inserting a wedge-like amphipathic helix into the membrane. The primary evidence for this mechanism is an increase in membrane bending capacity among a family of ENTH mutants with increasing hydrophobicity of the helix (Ford, Nature 2002). Since this discovery, amphipathic helices have been identified in diverse curved membrane structures including trafficking vesicles, viral buds, and multi-vesicular bodies. However, our recent work has demonstrated that membrane bending by ENTH does not require helix insertion. Specifically, whether ENTH attached to membranes by inserting a helix or using a histidine-NTA interaction, membranes became highly curved when ENTH covered 30% or more of the membrane surface. These results, coupled with analytical modeling, suggest that collisions among densely crowded membrane-bound proteins create steric pressure that drives bending (Stachowiak, Nature Cell Biology 2012). How can these seemingly conflicting results be reconciled? Here we report that increasing the hydrophobicity of ENTH's helix strongly increases the density of membrane-bound ENTH, suggesting that helix insertions drive bending by facilitating protein crowding. We correlate lifetime FRET measurements of the density of membrane bound proteins with membrane curvature measured using confocal and transmission electron microscopy, and find that all ENTH mutants drive bending when bound at high densities, regardless of helix hydrophobicity. This work resolves the conflict by demonstrating that the role of amphipathic helices in curving membranes is to create strong bonds between proteins and membranes, rather than to directly bend membranes through wedge insertion. This finding will impact understanding of the broad range of curved membrane structures in which helix insertion participates.

913-Plat
Lipid-Lipid Interactions Determine the Membrane Spontaneous Curvature
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The dominant view of how lipid chemistry determines membrane spontaneous curvature is through the ''shape'' of the lipid; a bilayer composed of lipids with small head groups will curve in to fit this space. However, the strength of lipid-lipid interactions may depend on curvature as it determines the relative orientation of neighboring lipids. The hypothesis of this work is that membrane spontaneous curvature is determined by these interactions.
This interaction view leads directly to non-additivity of the spontaneous curvatures of a lipid mixture. All-atom molecular simulations are presented that show non-additivity in the curvature preference of sphingomyelin/dioleoylphosphatidylethanolamine mixtures, as well as of liquid-ordered and liquid-disordered ternary mixtures.
The implications for biological processes are, first, that there is a new mechanism of localizing enhanced lipid compositions to particular sites (i.e., ordered phases form where their curvature is optimized), and second, that the energetic penalty to local Gaussian curvature (dominant in fusion pores) is less than the current literature suggests, as lipid-lipid interactions are stabilized by correlating with local curvature. These effects are entirely absent in mean-field and shape models of the spontaneous curvature.

914-Plat
Liposome Adhesion Generates Contractile Traction Stresses
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