endocytosis and exocytosis, and protein trafficking. Cholesterol-rich lipid domains have been hypothesized to exist in a liquid-ordered phase and play an important role in cellular functions. Here, we test the hypothesis that cholesterol diffuses as a complex with other lipids in a bilayer. In addition, we examine the role of lipid variation and proteins on the biophysical properties of biomembranes using comparative studies of giant unilamellar vesicles (GUVs) and plasma membrane vesicles (GPMVs) isolated from Hs578Bst live cells. The fluorescence dynamics assay used here includes two-photon fluorescence lifetime imaging, fluorescence resonance energy transfer, and diffusion (both rotational and translational). Different fluorescent lipid analogs are used in these studies to probe both the hydrophobic core and the head group region. Our comparative studies on GUVs and GPMVs serve as a platform to test our understanding of lipid-lipid and lipid-protein interactions in these biomembrane models.

2819-Plat
Two-dimensional Calorimetry: Imaging Thermodynamics and Kinetics of Phase Transitions of Biological Membranes
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Differential scanning calorimetry (DSC) is a relatively rapid and informative biophysical method for studying thermotropic phase behavior of biological membranes. More recently a pressure perturbation calorimetry has been introduced. The latter method is capable of characterizing membrane thermal volume expansion coefficient and kinetics associated with the phase transition. Notably, pressure perturbation calorimetry requires both pressure jump accessory and, most importantly, fast and sophisticated temperature control system to ensure constant sample temperature upon the pressure jump. Here we describe a calorimetry procedure and associated method of data analysis to characterize sample thermal properties as a two-dimensional (2D) object (temperature-time thermal image) whereas data obtained by conventional calorimetry are essentially one-dimensional. In brief, the method utilizes mathematical formalism of the Radon transform (back-projecting algorithm) to separate temperature and time dimensions from a series of thermal flux measurements obtained by a conventional DSC calorimeter at different scanning rates. By this manner static and time-dependent (i.e., relaxation) thermodynamic parameters of an object become resolved and displayed as a single 2D temperature-time thermal image. There are two main advantages of our 2D-calorimetry method: 1) 2D temperature-time thermal image separates and characterizes equilibrium and non-equilibrium thermal properties of a sample; 2) the method improves signal-to-noise ratio for conventional DSC measurements of equilibrium heat capacity as a function of temperature. We demonstrate these advantages of the 2D calorimetry method on examples of imaging thermodynamics and heat relaxation properties of lipid bilayers composed from single and mixed phospholipids with and without cholesterol. We also show that confining lipid bilayers inside nanopores of ca. 175 nm in diameter results in heterogeneous heat transfer kinetics while conventional equilibrium calorimetry curves remain unperturbed.
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2820-Plat
Raft recruitment of Membrane Proteins by Native Ligands and GPI-Anchored Proteins: A Model Membrane Study
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The role of cholesterol/sphingolipid-enriched lipid heterogeneities, also known as lipid rafts, in membrane protein function represents one of the fascinating topics in cell biophysics and cell biology. Progress in this research area has been slow, due to the small size and transient character of such heterogeneities in plasma membranes of resting cells. To overcome these experimental complications, here we describe two-types of protein-induced membrane protein recruitment processes to/from raft-like domains using a model membrane platform based on a polymer-tethered phospholipid bilayer. The domain-specific quantification of reconstituted membrane proteins in the bilayer is achieved using a combined setup of epifluorescence microscopy and confocal fluorescence correlation spectroscopy. In the first case, we present experimental data concerning the raft recruitment of membrane proteins due to the binding to their native ligands [receptor/ligand pairs investigated: FcγRIII/IgA; urokinase plasminogen activator receptor (uPAR)/ urokinase plasminogen activator (uPA); $\alpha_V\beta_3$ integrin/vitronectin; and $\alpha_5\beta_1$ integrin/fibronectin]. Interestingly, the ligand binding process causes substantial translocations among GPI-anchored proteins (FcγRIII and uPAR) and membrane-spanning receptors ($\alpha_V\beta_3$ and $\alpha_5\beta_1$) to/from raft-like domains. Remarkably, under the experimental conditions chosen, no ligand-induced receptor oligomerization can be observed. In the second case, we explore the impact of uPAR-integrin complex formation on the affinity of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ for lipid rafts. The uPAR/integrin complexation is determined for different ligand states using single molecule fluorescence microscopy. Importantly, our results confirm that such complexes enhance the affinity of integrins for raft domains, thus suggesting a mechanism of GPI protein-induced recruitment of transmembrane proteins to raft domains. Finally, the impact of monolayer and bilayer-spanning raft-mimicking domains on protein recruitment processes is discussed.

2821-Plat
Electromechanical Forces and Flexoelectricity in Plasma Membranes
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Flexoelectricity is a phenomenon analagous to piezoelectricity which describes the evolution of electric fields in two dimensional liquid crystals in response to curvature changes (and, conversely, changes in curvature due to applied electric fields). It provides a mechanism for cellular electromechanical transduction in flexoelectric membranes, a process which is important to the physiology of outer hair cells of the inner ear and touch sensitive nerve cells.
We have developed a simple electrostatic model for a bilayer containing anionic phospholipids. Charged ions surrounding the bilayer are distributed according to the Poisson-Boltzmann equation. The correct way to treat the adsorption of positive ions into the bilayer (the ''Stern layer'' of Gouy-Chapman-Stern theory) is not clear. Different regimes are investigated, including a Langmuir Isotherm and a fixed chemical potential approach. It is found that the presence/absense of a flexoelectric effect is critically dependent upon how this adsorption is handled.
Additionally, recent experimental work conducted on cell membrane tethers which are extended in an optical trap setup provides direct estimates for the tensile forces in these tethers as a function of applied voltages. We describe a model which gives quantitative agreement with these measurements.

2822-Plat
Characterizing Changes In The Structure And Orientation Of Supported Model Membranes Upon Binding Of Cholera Toxin B
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Cholera is caused by a protein toxin secreted by the bacterium *Vibrio cholerae*. This toxin is a member of the AB_5 superfamily of toxins composed of an active (A) and a binding (B) pentameric unit. The five binding subunits of the pentamer bind specifically to the GM_1, which is present in the outer leaflet of the host's plasma membrane; however, the mechanism for the translocation of the toxin is not well understood. Model membranes consisting of DMPC+cholesterol+GM_1 were supported on gold electrode surfaces. The changes in the structure and orientation of the model membrane upon binding of the cholera toxin B unit were explored using differential capacitance, chronocoulometry and polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). Changes in the structure and orientation of the toxin protein with respect to a changing electric field were similarly investigated. The IR data suggests that the binding of the toxin to the membrane causes a decrease in the number of *gauche* conformers, which is caused by the constriction of the acyl chains due to interactions between the toxin and the GM_1 glycolipids. The bound toxin induces some minor defects in the membrane; however, these defects are not significant enough to cause measurable changes in the average orientation of the membrane lipids. The major change in the bilayer upon binding of the cholera B unit was a remarkable decrease in the relative hydration of the membrane. This decrease in hydration is mostly like due to the separation of the bilayer from the aqueous electrolyte by the presence of the bound protein layer on the membrane surface. This work is part of ongoing study to understand the mechanism for translocation of the cholera toxin across the plasma membrane.

2823-Plat
COPI Coat Assembly Occurs on Liquid Disordered Domains and the Associated Membrane Deformations are Limited by Membrane Tension
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Cytoplasmic coat proteins are required for cargo selection and budding of tubulovesicular transport intermediates that shuttle between intracellular