

When phospholipid membranes are exposed to electric fields a variety of phenomena can be observed, such as phase separation, domain movement, electroporation, -deformation, -fusion, and -striction to name but a few. Understanding such responses is of both fundamental interest as well as of practical application.

Various thermodynamic susceptibilities of lipid membranes increase strongly in the melting transition, leading to large changes in, for instance, membrane conductivity, compressibility, bending elasticity, relaxation time, and geometry. Another such property (susceptibility) of the membrane is its electrical capacitance. In the phase transition both area and thickness change significantly, but also the dielectric coefficient can increase due changes in membrane composition. This coupling of the membrane's capacitance to its phase state implies that transient currents will appear if the membrane is pushed into the phase transition by changes in e.g. pH, membrane potential, pressure or temperature. On this poster we will show some of these phenomena, and discuss them in the context of the recently proposed soliton model of nerve signal propagation by Heimburg and Jackson, where the coupling between the electrical aspects and the phase state of the system is of central importance.

1438-Pos

FRET Reveals Coexisting Nanoscopic Fluid Phases in POPC/DSPC/Cholesterol

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Ternary lipid bilayer systems containing dioleoylphosphatidylcholine (DOPC) or diphytanoylphosphatidylcholine (DiPhyPC) as the low melting temperature lipid yield remarkably consistent phase diagrams when probed by methods with a wide range of spatial and temporal sensitivity. The resulting phase diagrams invariably show a large region of fluid/fluid phase coexistence at biologically relevant compositions, and have generated considerable interest as a potential explanation for lipid raft phenomena observed in plasma membrane. However, the unusual lipids DOPC and DiPhyPC are rare in mammalian plasma membranes. In contrast, phase diagrams with the biologically abundant palmitoyl-oleoylphosphatidylcholine (POPC) as the low melting lipid have mixed interpretations: studies using methods like fluorescence anisotropy and quantum yield (which have nanometer spatial resolution) report fluid/fluid coexistence that microscopy studies fail to detect. An explanation of these results in terms of first-order phase coexistence with nanometer-sized phase domains has proven controversial in the absence of a known mechanism for limiting lipid domain size. We show that the compositional dependence of FRET in the ternary systems DOPC/DSPC/cholesterol and POPC/DSPC/cholesterol is remarkably similar, and can be interpreted as arising from probe partitioning between phase domains. In addition, we present a quantitative model for the dependence of FRET efficiency on domain size and demonstrate its applicability to these systems.

1439-Pos

Quantitative IR Spectroscopy Studies of Changes in Lipid Dynamics and Organization in Isolated Stratum Corneum Exposed to Basic pH

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The outer layers of the epidermis, the stratum corneum (SC), provide the barrier function that is essential to life, primarily through the extracellular lamellar lipid matrix. Previous IR spectroscopy studies of isolated SC have shown the presence of ordered lipid bilayers, packed in orthorhombic and hexagonal domains. This lipid organization is essential to the barrier function of SC. In the current work we have investigated the effect of basic pH on lipid organization in SC. The outer surface of skin is routinely subjected to pH 10 solutions when exposed to soaps during cleansing. This exposure to basic pH has been shown to result in reduced barrier function and can lead to clinical irritation of the skin. Using IR spectroscopy methods previously developed in our laboratories to study isolated SC, we have examined the effect of pH 10 exposure on lipid organization in SC, monitoring both the intra- and inter-molecular lipid organization. The results of these studies show the T_m of SC lipids is significantly increased after pH 10 exposure. Furthermore, the change in bilayer T_m is not reversible. To explore changes in lipid packing underlying the pH-induced change in T_m , we are developing quantitative approaches evaluating changes in the amount of orthorhombic and hexagonal chain packing in normal and challenged SC. The results of these quantitative approaches to chain packing are being correlated to the changes in conformational order and increasing T_m after SC is exposed to pH 10. We will present our IR spectroscopic data showing irreversible increases in lipid T_m and the accompanying quantitative analysis of lipid packing changes.

1440-Pos

Phase Diagram of a 3-Component Lipid Mixture of PS/PE/CHOL to Model the Inner Leaflet of a Plasma Membrane

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The two leaflets of an animal cell plasma membrane are compositionally asymmetric: the outer leaflet has a relatively high concentration of phosphatidylcholine (PC) and sphingomyelin (SM), and the inner leaflet has most of this membrane's phosphatidylethanolamine (PE) and almost all of its phosphatidylserine (PS). The overall cholesterol mole fraction is high, with its distribution between the two leaflets uncertain. Whereas model membrane studies using lipids mimicking the outer leaflet composition have revealed complex mixing behavior including solid/liquid and liquid/liquid phase separations, the mixing behavior of the inner leaflet is still poorly understood. We have constructed a phase diagram for a model mixture of the inner leaflet using a high melting temperature PS, a low melting temperature PE, and cholesterol. Dipalmitoyl PS (DPPS) and palmitoyl-oleoyl PE (POPE) are in the solid (L_β) and liquid disordered (L_α) phases, respectively, at the experimental temperature of 30°C. A combination of fluorescence microscopy and fluorescence resonance energy transfer (FRET) between fluorescent lipid probes was used to map all regions of the phase diagram. An L_β/L_α coexistence region was observed up to at least 15% cholesterol.

1441-Pos

Lipid Monolayer Line Tension Measurements and Model Convolution

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Research into the phase separation of coexisting liquid phases in mixed phospholipid/sterol monolayer systems is an important experimental approach to understand the lateral inhomogeneities or "lipid rafts" within lipid membranes. We present measurements of line tensions between immiscible phases in mixed monolayer systems of phospholipids and the cholesterol analog 25-hydroxycholesterol. This hydroxycholesterol is an interesting modulation of the cholesterol structure for both its implicated pathological effect on the plasma cell membrane as well as its unique phase diagram. In addition to these experimental studies we will also discuss ongoing work to improve our line tension measuring tools. Model-convolution microscopy is a technique that can be used to assess the effectiveness of image processing routines by testing them against experimenter determined parameters. Here a theoretical model is used to generate the underlying structure of an image and this is convolved with the point spread function of light. We will also present results obtained using this technique to study the importance and necessity of the incompressibility constraint in the Fourier analysis of lipid domains.

1442-Pos

The Complex and Unexpected Ionization Behavior of Phosphoinositides

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The phosphorylated forms of phosphatidylinositol, among all minor membrane lipid species, are arguably the most important in the regulation of intracellular signaling processes. Specificity is achieved by the selective phosphorylation of the inositol headgroup, which can carry a total of three phosphomonoester groups. Many proteins have developed special binding domains that facilitate specific binding to particular phosphoinositide species, while other proteins interact with phosphoinositides via nonspecific electrostatic interactions. Here we describe the ionization properties of all three naturally occurring bisphosphates as well as phosphatidylinositol 3,4,5-trisphosphate in model lipid membranes composed of phosphatidylcholine. We find substantial differences in ionization behavior between the three bisphosphates, and the ionization behavior of the triphosphate is extraordinarily complex, indicating the crucial role of phosphate substitution pattern. The results are explained by intramolecular hydrogen bonds in the headgroups of the individual phosphatidylinositol polyphosphates. Surprisingly however, we also find evidence for intermolecular hydrogen bond interactions, suggesting that e.g. PI(4,5)P₂ can cluster in model membranes. Additionally, we investigated the effect of other major membrane lipid species on the ionization properties of PI(4,5)P₂, specifically cholesterol and phosphatidylethanolamine. Preliminary results are discussed in terms of possible intermolecular interactions.

1443-Pos

Effect of Polymer on the Elastic Properties of Membranes

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Macromolecules interacting with membranes can modify physical properties of the latter such as the bending rigidity or their local topology. The addition of a