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 palmitoyloleoylphosphatidylcholine (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/chol and POPC/DSPC/chol 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 palmitoyloleoyl 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 trisphosphate 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. $PI(4,5)P_2$ can cluster in model membranes. Additionally, we investigated the effect of other major membrane lipid species on the ionization properties of $PI(4,5)P_2$, 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

water-soluble polymer, PEG, to the lamellar phase of DMPC or of several surfactants, induces a topological transformation to a vesicular phase. Such transition can be understood in terms of a modification of the elastic properties of the membranes. In this work we perform a dynamic light scattering (DLS) study of the effect of PEG on phospholipid and surfactant bilayers. The experimental results show that the addition of the polymer slows down the dynamics of the membranes. From fits to an available theory for the scattering of dilute membrane systems, we have calculated the bending elastic modulus of the bilayers. This modulus increases with increasing polymer concentration, thus confirming that the macromolecule modifies the elastic properties of the membranes.

Membrane Active Peptides II

1444-Pos

Effects of a Membrane-Active Amphibian Antimicrobial Peptide on the Bacterial Proteome

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Ribosomally synthesized antimicrobial peptides (AMPs) are conserved components of the innate immunity of all life forms and represent the most ancient and efficient weapon against microbial pathogens.

The emergence of multidrug-resistant microbes has urgently required the discovery of new antibiotics with a new mode of action, and AMPs represent promising candidates. Intense research focusing on AMPs is currently directed to the elucidation of their mode(s) of action.

Nevertheless, very little is known about their effects on intact bacteria.

Here we report on Esculentin 1-18 [Esc(1-18)], a linear peptide covering the first 18 N-terminal residues of the full length amphibian peptide esculentin-1b. Esc(1-18) retains the antimicrobial activity of esculentin-1b against a wide range of microorganims, with negligible effects on mammalian erythrocytes. To expand our knowledge on the molecular mechanism underlying the antimicrobial activity on Gram-negative bacteria, we investigated the effects of this peptide on *Escherichia coli*, by studying its: i) structure in membrane mimicking environment; ii) killing kinetic; iii) bactericidal activity in different media; iv) ability to permeate both artificial and bacterial membranes; v) capacity to synergize with conventional antibiotics; vi) effect on cell morphology and proteome by means of electron microscopy and proteomic techniques, respectively.

These studies have indicated that Esc(1-18) (i) kills *E. coli* via membrane-perturbation; (ii) elicits identical changes in the bacterium's protein expression pattern, at both lethal and sub-lethal concentrations; and (iii) preserves antibacterial activity under conditions closer to those encountered *in vivo*. This is in contrast with many host defence peptides that kill microorganisms by altering intracellular processes and lose activity in physiological solutions. Importantly, to the best of our knowledge, this is the first case showing the effects of an amphibian AMP on the protein expression profile of its bacterial target.

1445-Pos

A New Look at an Old Friend: Novel Insights Into Pore Formation by Alamethicin

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Alamethicin is a 20-amino acid long antibiotic peptide produced by the fungus *Trichoderma viride.* In the literature, alamethicin is the most commonly cited example of a barrel-stave model pore-forming peptide. In this model, amphipathic peptides form a long-lived transmembrane pore by aligning hydrophobic and hydrophilic residues with the lipid bilayer and aqueous pore respectively. It has been reported that voltage-independence of alamethicin pores relies on salt and peptide concentrations. We have developed a set of fluorescence-based assays for leakage, stable pore formation and lipid flip-flop using large unilamellar vesicles (LUVs) to help define the mechanism and potency of pore forming peptides. An additional pre-incubation assay differentiates dynamic pores from long-lived pores. When applied to alamethicin, our suite of assays show that, at 2.0 μM peptide, alamethicin forms voltage-independent pores in anionic or zwitterionic vesicles at peptide-to-lipid ratios as low as 1:2000. Less than 20 peptides per vesicle is sufficient to allow for complete vesicle permeabilization. Even after overnight incubation with vesicles, alamethicin promotes continuous lipid flip-flop, and is able to permeabilize multiple additions of new vesicles. Other pore forming peptides tested at this P:L ratio do not promote continuous flip-flop and do not exchange into new vesicles. We postulate that, unlike other pore-forming peptides, which mostly behave like classical barrel-stave pores, alamethicin is in a continuous dynamic equilibrium between transmembrane, interfacially bound and aqueous forms.

1446-Pos

Effect of L- to D-Peptide Isomerisation on the Activity of Antimicrobial Peptide Anoplin

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Isolated from the venom sac of solitary spider wasp, Anoplius samariensis, Anoplin is the smallest linear α-helical antimicrobial peptide found naturally up to date. It has broad spectrum activity against both Gram-positive and Gram-negative bacteria, and little hemolytic activity toward human erythrocytes (1,2). Previous studies showed that substitution of all amino acids in the peptide with D-isomers can increase the bioavailability and reduce peptide degradation without affecting the antimicrobial properties since the net charge and the hydrophobicity are retained (3). In the present work, two stereoisomers of Anoplin were studied using UV resonance Raman spectroscopy, Langmuir Blodgett, atomic force microscopy, calcein leakage assay and antimicrobial assay. UV resonance Raman data indicate that the two forms of the peptide adopt similar conformations in aqueous buffer and in membrane mimicking solutions. Monolayer isotherms show that D-Anoplin has a lightly greater area per molecule than L-Anoplin. Finally, membrane rupturing ability of both stereoisomers was found to depend strongly on membrane composition.

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1447-Pos

Biophysical Studies of Cecropin-Mellitin Antimicrobial Peptides with Improved Selectivity

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Antimicrobial peptides (AMPs) have received much attention as models for the development of antibiotics capable of meeting the challenge of drug-resistant infections. Our research has focused on a linear, 15-residue cecropin-mellitin (CM) hybrid peptide designated CM15. In these studies we compare the biological activities and membrane interactions of CM15 and lysine substituted derivates designed to have optimized amphipathicity upon membrane binding. Previous studies have shown that these lysine enriched peptides maintain the high antimicrobial activity of the parent peptide but have substantially decreased hemolytic effects (Sato and Feix, Antimicrob. Agents Chemother. 52, 4463, 2008). Using model membranes to understand the differences that govern peptide-membrane interaction, we have performed a series of biophysical experiments including circular dichroism (CD), fluorescence, and site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy. These experiments establish the structures of the membrane-bound peptides, determine the extent of membrane lysis, and allow determination of partition coefficients and depth of penetration. Our results show that differences in red cell hemolysis can be reconstructed using model membranes, and provide insights into the mechanism of membrane disruption. This work was supported by award number R01GM068829 from the National Institute Of General Medical Sciences.

1448-Pos

Structural Modifications to Convert Melittin from a Cytolytic Peptide to a Stable Cargo Linker

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Melittin is a 26 amino acid peptide that comprises more than half of the dry weight of the venom of the honeybee Apis Mellifera. In previous studies, we demonstrated that melittin stably bound to the lipid membrane of perfluorocarbon (PFC) nanoparticles and served as an active anti-cancer therapeutic agent *in vivo (Soman et al. J Clin Invest. 2009)*. Here, we report the structure modification that converts the mellittin to a cargo linker for **post-formulation** liposome customization. We introduced point mutations and truncations to define the lytic and membrane binding activities of melittin. For each of the mutations, lytic activity of the peptides was tested by the carboxyfluorescein fluorescence dequenching assay on carboxyfluorescein encapsulated liposomes. Among all six melittin mutations, the mutation, D1-7, with the first 7 amino acids removed (VLTTGLPALISWIKRKRQQ) dramatically reduces the melittin lytic activity. Using immobilized Giant Unilamellar Vesicles (GUV), we illustrated that at peptide:lipid ratio of 40:1, D1-7 bound to the GUV without pore forming, while at peptide:lipid ratio of 1:83, melittin already formed pores on the GUV