depending on how the balance of attractive and repulsive force is shifted by the nature of the aqueous solution. We performed small angle x-ray scattering measurements to reveal how the buffers modify lipid interactions. Buffers loosely associate with the lipid membrane and alter their surface charge causing the MLVs to swell. Interestingly, as opposed to monovalent salts which charge up the PC membranes negatively, MOPS charges PC membranes positively. We have used small angle x-ray scattering to measure the modification of membrane forces and we have measured the diffusion of lipid aggregates in electric fields to determine the charging effect of the buffers on PC membranes. By measuring how buffers modify the electrical state of lipid membranes we can better understand how buffers behave at the interface of biological membranes.
[1] H. I. Petrache, T. Zemb, L. Belloni, and V. A. Parsegian. *Proc. Natl. Acad. Sci.*, 2006, 103:7982-7987.

1431-Pos
Molecules Pushing Molecules: Dynamic Consequences of Crowding
Philip A. Gurnev[1], Rudolf Podgornik[2], Joel A. Cohen[3], Sergey M. Bezrukov[1], V. Adrian Parsegian[4].
[1]National Institute of Child Health and Human Development, Bethesda, MD, USA, [2]Department of Physics, Faculty of Mathematics and Physics, University of Ljubljana, and Theoretical Physics Department, J. Stefan Institute, Ljubljana, Slovenia, [3]Department of Physiology, A. A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA, [4]Department of Physics, University of Massachusetts, Amherst, MA, USA.
Membrane pores, such as alpha-hemolysin, sieve molecules to provide passage. Large polymers are excluded while monomers and small polymers can pass. At high concentrations, flexible polymers lose their size that exists under dilute conditions. Rather, flexible polymers look more like strings with regions of limited coherence. This transition is clear from the shift in osmotic pressure vs. polymer concentration: van't Hoff regime in the dilute limit but des Cloizeaux regime at higher concentrations. Under crowded conditions, a polymer previously unable to enter the alpha-hemolysin pore suddenly enters when the apparent limiting size is in the region of limited coherence. Mixtures of small and large polyethylene glycols show exclusion in this range where the larger species exert stress that drives the smaller polymers into and across pores at concentrations far larger than those in the bathing solution. This coupling of polymer activities and consequent conferred mobility creates a new form of crowding-driven transport.

1432-Pos
Effect pf PAH Concentration on Sops Liposomes
Ricardo L. Esparza[1], María Leticia Valenzuela Sánchez[1], Araceli Arteaga Jiménez[2], César Márquez Beltrán[3], Amir Maldonado Arce[1].
[1]Universidad de Sonora, Hermosillo, Mexico, [2]Universidad Autónoma de Puebla, Puebla, Mexico, [3]Universidad de Sonora URN, Caborca, Mexico.
We have prepared SOPS liposomes using the hydration technique. Optical microscopy experiments show that the size and shape of the liposomes do not change when they are swell with a glucose/sucrose solution. To the SOPS liposome system we add the polyelectrolyte PAH (Poly-Allylamine Hydrochloride), producing a drastic change in the liposome structure. We have studied the influence of PAH on the liposome shape and size distribution by means of Differential interference contrast microscopy (DIC). The results show that PAH interacts with the SOPS liposomes forming PAH-SOPS complexes.

1433-Pos
Localized Photothermal Heating of Temperature Sensitive Liposomes
Natalie A. Forbes, Joseph A. Zasadzinski.
University of California, Santa Barbara, Santa Barbara, CA, USA.
A drug delivery system consisting of a temperature sensitive liposome coupled to hollow gold nanoshells allows precise spatial and temporal control of drug release. A small fraction of lysolipid in a primarily dipalmitoylphosphatidylcholine (DPPC) liposome lowers the membrane transition temperature to that obtainable by mild hyperthermia, while simultaneously enhancing the membrane permeability at the transition temperature. Hollow gold nanoshells coupled to the liposomes heat the membrane when irradiated by a continuous wave near-infrared laser. The heat generated by the nanoshells can be tuned to control local membrane temperature, and hence the membrane permeability and rate of drug release. This system could be used to deliver anticancer drugs directly to a tumor site. Additionally, the ability to correlate drug release with membrane temperature allows us to empirically determine the local heat generated by the hollow gold nanoshells upon laser irradiation.

1434-Pos
Phase Diagram of a 3-Component Lipid Mixture Containing a Polyunsaturated Phosphatidylcholine
Tatyana Konyakhina, Gerald W. Feigenson.
Cornell University, Ithaca, NY, USA.
Polyunsaturated acyl chains have special influences on the mixing and phase behaviors of lipid mixtures. Their high degree of unsaturation affects the physical properties of biomembranes in ways that are still not fully understood, and their high concentration in some membranes makes them key players in membrane structure. We are investigating the 3-component phase diagram for the biologically relevant mixture of brain-SM/ 18:0-22:6 PC/ cholesterol. Fluorescence microscopy imaging of giant unilamellar vesicles (GUVs) was employed for phase boundary visualization and phase identification of the 3-component mixture. Fluorescent lipid probes having complementary partitioning behavior are used in FRET measurements to enable more quantitative analysis. Of particular interest is the region of Lo + Lα phase coexistence, which shows macroscopic phase separation.

1435-Pos
Investing Early Signaling Events in IgE-FcεRI Activation Using SEM
Ethan N. Chiang, Sarah L. Veatch, David Holowka, Christopher Ober, Barbara A. Baird.
Cornell University, Ithaca, NY, USA.
Antigen-mediated cross-linking of immunoglobulin E (IgE) bound to its high affinity receptor FcεRI on mast cells initiates a transmembrane signaling cascade that results in cell activation and exocytotic release of chemical mediators involved in allergic response. Plasma membrane lipids and proteins redistribute as part of this transmembrane signaling process. To understand the functional role of these redistributions, resolution of their size, composition and structure on the nanometer scale is required. We utilize high resolution scanning electron microscopy (SEM) to directly visualize sub-micron membrane domains in intact cell membranes. In our experiments, the distribution of gold-labeled proteins and lipids is analyzed at the surface of intact fixed cells using backscattered electron detection. In parallel, we also observe membrane topography using secondary electron detection. We use a pair-correlation function analysis to quantify protein distributions and parameterized domain size. We have mapped the distribution of a variety of proteins, both related and non-related to the IgE signaling pathway. Using this experimental and quantitative method, we observe dramatic changes in the nano-scale membrane distribution of IgE due to stimulation with multivalent ligands. In resting cells, IgE receptors are clustered into small domains less than 30nm. After stimulation, receptors redistribute into large domains that are correlated at long length-scales and subsequently reduce in size at long stimulation times. We also observe cross-linking-dependent rearrangement of several inner leaflet-associated proteins implicated in early signaling events. In contrast, outer leaflet GPI-anchored proteins are not affected. We have also quantified the co-redistribution of IgE with other membrane proteins after stimulation using cross-correlation functions. These findings provide valuable insights into the mechanisms that drive the selective nanoscopic reorganization of plasma membrane proteins during immune cell signaling.

1436-Pos
Fluorescence Measurements in Fruit Fly (Drosophila Melanogaster)
Lin Shin Teo.
National University of Singapore, Singapore, Singapore.
Drosophila melanogaster is a widely used animal model in developmental biology. In *Drosophila*, the wealth of genetic tools allows expression of any given marker or construct in specific cells or tissues within the organism. This is especially advantageous since particular cells can be studied in their natural 3D organization, avoiding possible artefacts and deviations from the physiologically relevant situation that may be introduced in cell cultures. For the study of molecular dynamics within cells and cell membranes on a single molecule level, we performed fluorescence correlation spectroscopy (FCS) within the *Drosophila* embryonic nervous system. Using a GAL4 driver expressed in a small subset of neurons, we expressed fluorescently tagged fusion membrane proteins, CD8 and flotillins-2, in two identified motor neurons per hemisegment of the embryonic central nervous system (CNS). We obtained autocorrelation curves for membrane and cytoplasmic probes which show diffusion times that correspond to their respective subcellular locations. By additionally expressing (non-tagged) proteins which influence lipid metabolism, example ceramidase, we are able to follow changes in the molecular dynamics of membrane proteins. With this approach, we are studying the biophysical properties of the cellular membrane *in vivo* and *in situ* and will extend this in the future to different genetic backgrounds. The study shows that *in vivo* analyses provide us greater insights into the role of membrane dynamics in the context of development, differentiation and pathogenesis of diverse diseases.

1437-Pos
The Influences of Electric Fields on Lipid Membranes
Andreas Blicher, Thomas Rainer Heimburg.
Niels Bohr Institute, Copenhagen, Denmark.

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
Frederick A. Heberle, Gerald W. Feigenson.
Cornell University, Ithaca, NY, USA.
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
Peter Saad[1], Daniel Moore[2], David J. Moore[2], Evan Flach[1], **Carol R. Flach[1]**, Richard Mendelsohn[1].
[1]Rutgers University, Newark, NJ, USA, [2]International Specialty Products, Inc., Wayne, NJ, USA.
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
Nelson F. Morales-Penningston, Shih Lin Goh, Gerald W. Feigenson.
Cornell University, Ithaca, NY, USA.
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
Andrew H. Nguyen[1], Erkan Tuzel[2], Benjamin L. Stottrup[1].
[1]Augsburg College, Minneapolis, MN, USA, [2]Worcester Polytechnic Institute, Worcester, MA, USA.
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
Edgar E. Kooijman, Katrice E. King, Mahinda Gangoda, Arne Gericke.
Kent State University, Kent, OH, USA.
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
Ramon Iniguez, Heriberto Acuna, **Amir Maldonado**.
Universidad de Sonora, Hermosillo, Mexico.
Macromolecules interacting with membranes can modify physical properties of the latter such as the bending rigidity or their local topology. The addition of a