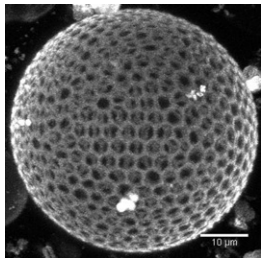
Membrane Physical Chemistry II

2731-Pos Board B717
Thermal Stress of Supported Lipid Bilayer Induces Formation and Collapse of Uniform Radius Tubules
Kimberly L. Weirich, D. Kuchnir Fygenson.
Lipid bilayer morphologies and the transitions between them are important to many cellular processes. Supported lipid bilayer (SLB) provides a model system in which to quantitatively investigate transitions from planar to tubular and tubular to spherical morphologies. Following a small increase in temperature (~5-10°C) flexible filaments extrude from a fluid SLB. Individual filaments can reach hundreds of microns in length before spontaneously collapsing into discs. We demonstrate that the filaments are tubular by decreasing the external buffer concentration, which causes them to swell, first into resolvable tubules with capped ends and then into giant vesicles. At high ionic strength, the sub-resolution tubules are adsorbed to the SLB, enabling the measurement of their radius to within ± 5 nm using conventional fluorescence microscopy. The radius depends on the lipid tail composition and varies $<10\%$ along the tubule length. Under tension, tubules are even more uniform, having no measurable variation in radius.

2732-Pos Board B718
Lipase Action on Self-Assembled Lipid Liquid Crystalline Nanoparticles
Justas Barauskas, Daniel Anderberg, Allan Svendsen, **Tommy Nylander**.
Natural lipase substrates are supramolecular assemblies, either emulsion droplets or liquid crystalline, aggregates. Most of the lipolysis takes place at interfaces and is dependent on the structure and organization of the lipid/water boundary as well as internal structure of the aggregate. The internal structure control both the access of the susbstrate as well as the ability to take care of the product. During the course of the reaction, hydrolysis products interact with lipid substrate, continuously change the interface and aggregate characteristics. Most of the studies so far have been focused on the changes of that take place at the lipid aqueous interface. We have used non-lamellar self-assembled lipid nanoparticles as well defined and biomimicing substrate in order to gain fundamental knowledge on lipase (Thermomyces lanuginosus lipase) catalyzed processes in terms of the changes in morphology and internal structure of the lipid aggregate (substrate). The results from two types of substrate structures will be presented, namely glycerol monooleate -based cubic and reversed hexagonal liquid crystalline nano-particles. These types nano-particles are likely to occur during the lipolysis process and also have potential use as drug delivery vehicles. The changes in lipid aggregate morphology and internal structure as a function of a lipase action have been investigated by means pH-stat titration, cryogenic transmission electron microscopy (cryo-TEM) and synchrotron X-ray diffraction techniques. In the cryo-TEM study we also used lipase conjugated to gold nanoparticles, enzymatically active hybrid nanoparticles, to simultaneously visualize the location of the enzyme and the effects of enzymatic digestion of lipid aggregates. Our results clearly show that the enzyme action is strongly influenced by the self-assembled structure and the lipid composition providing the possibility to control lipase activity.

2733-Pos Board B719
Vesicle and Lipid Bilayer Dynamics: Cross-Linking Effects and FRAP Analysis
Michael Kessler, Robin Samuel, Katerine Baldwin, Rahul Gupta,
Arthur Lee, Susan D. Gillmor.
We investigate the effects of perturbations on lipid phase dynamics. Our primary tools are confocal microscopy and differential scanning calorimetry (DSC). In coexisting fluid two-phase vesicles we have characterized cross-linking in the fluid disordered phase. Instead of reaching thermodynamic equilibrium, we have documented an increase in meta-stable configurations. Using fluorescence recovery after photobleaching (FRAP), we investigate how cross-linking affects diffusion in lipid bilayer. The diffusion perturbations reveal that cross-linking and non-specific binding slows lateral mobility, which alters lipid dynamics. Since cell membranes are not at thermodynamic equilibrium, our investigations into the dynamics behavior are pertinent to understanding membrane response to common events, such as receptor-ligand complexing, glycosylation, and receptor platform formation.



2734-Pos Board B720
Modulated Phases in 4-Component Lipid Mixtures
Jonathan J. Amazon, Jing Wu, Frederick A. Heberle, Gerald W. Feigenson.
The 3-component lipid mixture DSPC/POPC/CHOL exhibits a rich equilibrium compositional phase diagram. When a fourth lipid component, DOPC, is titrated in to replace POPC, unusual phase separation emerges in a portion of the composition space. Elongated stripe and honey comb like structures of coexisting liquid ordered and liquid disordered phases are observed in giant unilamellar vesicles. GUV images were examined by fluorescence wide field, 2-photon, and confocal microscopies. The specific mechanisms by which this phase modulation is driven are not yet well understood. The work of Seul and Andelman suggests that the formation of these structures may be driven by multiple order parameters with competing interactions. We analyzed many different order parameters and interactions using the formalism of field theory, diffusion kinetics, and computational modeling to better characterize the microscopic details of this novel phase behavior.

2735-Pos Board B721
Dynamic Critical Exponent for Concentration Fluctuations in a Lipid Bilayer
Aurelia R. Honerkamp-Smith, Benjamin B. Machta, **Sarah L. Keller**.
We present the first systematic measurement of the effective dynamic critical exponent z(eff) in a 2-dimensional system with conserved order parameter surrounded by a bulk 3-dimensional fluid, here a lipid membrane in water. We measure the dynamic structure function for concentration fluctuations in the membrane. We use dynamic scaling to collapse structure functions at different wavenumbers, thereby obtaining the effective dynamic exponent z(eff). We find that as the membrane approaches the critical temperature, z(eff) approaches 3, consistent with theoretical prediction [1]. Our result is fundamental to both biology and physics since membranes isolated from cells are poised near miscibility critical points [2].
References
[1] Mikko Haataja. Critical dynamics in multicomponent lipid membranes. Physical Review E 80, 020902 (2009).
[2] Sarah L. Veatch, Pietro Cicuta, Prabuddha Sengupta, Aurelia Honerkamp-Smith, David Holowka and Barbara Baird. Critical Fluctuations in Plasma Membrane Vesicles. ACS Chem. Biol., 3, 287-293 (2008).

2736-Pos Board B722
Miscibility of Ternary Membranes Containing Charged Lipids and Confirmation of Membrane Composition by Mass Spectrometry
Matthew C. Blosser, Cameron W. Turtle, Jordan B. Starr, Sarah L. Keller.
Here I present phase diagrams of vesicles containing phosphatidylcholine (PC), an uncharged lipid; phosphatidylglycerol (PG), a charged lipid; and cholesterol. I have found that this mixture exhibits coexisting liquid phases over a wide range of temperatures and compositions. I have found that miscibility in membranes containing charged lipids occurs over similar ranges of temperatures and lipid compositions as in membranes containing only uncharged lipids. Techniques for creating vesicle membranes containing charged lipids are significantly more challenging and less well characterized than for membranes containing neutral lipids. Here I use mass spectrometry to determine that the final membrane lipid composition is close to that of the initial stock solution. Specifically, I use a quadrupole spectrometer with an electrospray source using multiple reaction monitoring (MRM) in both positive and negative mode. Since the same instrument is used to quantify both the ratio of PC to PG and PC to cholesterol, the entire composition can be determined from one sample. The sensitivity of the technique is higher than phosphorus assays, and the MRM makes it extremely unlikely that signal is due to molecules other than the lipids of interest. This result confirms that vesicles made by gentle hydration have a predictable composition at both high and low fractions of charged lipids.

2737-Pos Board B723
Measurement of Late Stage Coarsening on Lipid Membranes
Cynthia A. Stanich, Aurelia R. Honerkamp-Smith, Gregory Garbès Putzel,
Thien-An D. Hua, Andrea K. Lamprecht, Christopher S. Warth,
Sarah L. Keller.
We investigate the diffusion and growth of liquid domains in the membrane of giant unilamellar vesicles (GUVs) composed of a ternary mixture of saturated phospholipids, unsaturated phospholipids, and cholesterol when the temperature is quenched below the miscibility transition temperature. After a period of nucleation (Lifshitz, et al., 2002), domains can grow by two mechanisms in the late stages of coarsening. These mechanisms are collision and coalescence of liquid domains and Ostwald ripening. Both contribute to the measured growth exponent, z, where domain radius, $R \propto t^z$. If the area fraction of one of the phases is small, the later stage of domain growth has been predicted by