restriction imposed by a supporting substrate. We investigate miscibility transition of ternary lipid mixture, DPPC / DOPC/ Cholesterol, using a combination of fluorescence imaging and time-resolved fluorescence anisotropy. The technique affords unprecedented dynamic characterization for lipid orientation, self-assembly, and dynamic freedom as the monolayer is forced from the liquid to the gel phase. We demonstrate the novelty and applicability of this device by contrasting the time-resolved fluorescence signal of three different lipid probes: 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] hexanoyl}-sn-glycero-3-phosphocholine (NBD-PC), 5-butyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-3-nonanoic acid (BIODIPY), and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Dil) which show dramatically different orientation and dynamic freedom when bound to the lipid layer, over a range of lipid phases. Using this technique we can resolve highly dynamic processes such as the insertion of peptide and proteins into the lipid membrane.

2569-Pos Board B339

Electrostatic Effects on Model Bilayer Stability and Structure

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Cellular membranes consist largely of phospholipids, making phospholipids important players for cell processes and cell-cell interactions. Electrostatics are postulated to control structure and function in lipid bilayers. While the effects are largely acknowledged, the mechanisms underlying electrostatic mediated processes are less clear. We have utilized Raman spectroscopy, surface-enhanced Raman scattering (SERS), laser transmission spectroscopy (LTS) and atomic force microscopy (AFM) to monitor the structure and chemical interactions that occur in the classic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine (DPPS) model system. Specifically, we are exploring the stability of these model bilayers in response to Ca^{2+} addition. Our results suggest that electrostatic and chemical interactions induce forces within the bilayer that result in destabilization of vesicles and domain formation in supported bilayer systems.

2570-Pos Board B340

Anomalous Freezing Behavior of Nanoscale Liposomes

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Experiments have shown that the melting transition of small liposomes is broadened when compared to large vesicles or planar membranes [1,2]. Despite their significant biological and biomedical importance, theoretical and computational studies of the phase behavior and structural properties of small liposomes have been limited [3,4]. We present here a systematic computational study of the phase behavior and structural properties of liposomes using a recently developed coarse-grained particle-based model [5]. We particularly focus on the effect of liposome diameter on their thermal and structural properties. Below the melting transition, liposomes are faceted with the gel facets separated by ''grain'' boundaries that are in the fluid phase. In agreement with experiments, we found that the melting transition is significantly broadened as the liposome diameter is decreased and that the heat capacity exhibits two distinct peaks for diameters less than 33 nm, an indication of a decoupling of the melting transition of the two leaflets. This decoupling is clearly demonstrated by the chain order parameters of the two leaflets, which show that the upper leaflet undergoes a melting transition before the inner leaflet. In the gel phase, the lipid tails of the inner leaflet are less ordered than those of the outer leaflet, and the discrepancy between the order parameters of two leaflets increases with decreasing liposomes' diameter. This work is supported by NSF grants (DMR-075547 and EPS-1004083).

[1] R.L. Biltonen and D. Lichtenberg, Chem. Phys. Lipids **64**, 129 (1993)
[2] H. Nagano, T. Najanishi, H. Yao, and K. Ema, Phys. Rev. E **52**, 4244 (1995)
[3] P.V. Ivanova and T. Heimburg, Phys. Rev. E **63**, 041914 (2001)
[4] H.J. Risselada and S.J. Marrink, Soft Matter **5**, 4531 (2009)
[5] J.D. Revalee, M. Laradji, and P.B. Sunil Kumar, J. Chem. Phys. **128**, 035102 (2008)

2571-Pos Board B341

Membrane Structure of Small Unilamellar Vesicles Determined by Small Angle X-Ray Scattering: A Comparison of Full and Local Spectrum Fittings

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We determined the membrane structure of small unilamellar vesicles in aqueous solution by using small angle X-ray scattering. The multi-parameter symmetry and asymmetry models were constructed to fit the full spectrum scattering curve to determine the membrane structure. As a comparison, a simple model with one parameter was used to fit the scattering curve around the second peak to obtain the membrane thickness. We used the model membranes composed of saturated and unsaturated lipids with different chain lengths to prepare small unilamellar vesicles in aqueous solution. The X-ray light source in BL13A and BL23A beam lines of NSRRC and home-made temperature controlled cell with Mylar windows will be applied in the measurements. Consequently, the membrane thicknesses extracted from full and local spectrum fittings are consistent and in agreement with reference papers. However, the parameters determined by full spectrum fittings exist discrepancies in different models except for membrane thickness. The result suggests that one parameter fitting of local spectrum should be a simple, reliable and efficient way to determine membrane thickness of unilamellar vesicles in aqueous solution.

2572-Pos Board B342

Molecular Structure of Phosphatidylglycerol Bilayers: Fluid Phase Lipid Areas and Bilayer Thicknesses as a Function of Temperature

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We have determined bilayer structural parameters of commonly used phosphatidylglycerols (PGs) in the fluid phase, by simultaneously analyzing small-angle neutron and X-ray scattering data. We report the temperature dependence of bilayer parameters obtained using the scattering density profile (SDP) model, including the area per lipid and overall bilayer thickness, as well as various intrabilayer structural parameters (e.g. hydrocarbon region thickness). Lipid areas were found to be larger than their neutral phosphatidylcholine (PC) counterparts, which is likely due to electrostatic repulsion of PG headgroups. In general, PG and PC bilayers show a similar response to changes in temperature and chain length, but a differential effect is observed with regard to chain unsaturation: the inclusion of a double bond in a PG lipid results in a smaller change in bilayer area and thickness than for the corresponding PC lipid. The extrapolated molecular area of saturated PG lipids at infinite chain length is similar to that of PC and PE, indicating the pivotal role of the glycerol-carbonyl backbone in shaping the lipid-water interface.

2573-Pos Board B343

The Detailed Scattering Density Profile Model of Pg Bilayers as Determined by Molecular Dynamics Simulations, and Small-Angle Neutron and X-ray Scattering Experiments

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The complex dynamics exhibited by biological membranes are closely correlated to the membrane's structure. Accurate structural data regarding the various membrane components are therefore important in determining specific biomembrane functions. The binding free energy, for example, of Lactoferricin B to mammalian-like membranes (i.e. no net charge) and bacterial-like membranes (i.e net negative charge) has been predicted from molecular dynamics (MD) simulations. However, for the simulation to make any kind of prediction, an accurate structure of the membrane lipids is needed. Area per lipid is often used as the key parameter when assessing the validity of MD simulations. On the other hand, lipid areas obtained from experiment have used models and are thus model dependent. It has therefore been proposed that a better test for validating MD simulations is to compare them to ''raw'' experimental data (e.g. in form of scattering form factors). Experimentally obtained scattering form factors then become the basis for the synergy between experiment and simulation, whereby the simulation results guide the development of more realistic models, and in turn, experimental data aid in the development of more accurate MD force fields.

We combine MD simulations and experiment, both small-angle neutron (SANS) and small-angle X-ray scattering (SAXS), to determine the precise structure of bilayers comprised of bacterial-like (phosphatidylglycerol, PG) lipids. Experiment and simulation are used to develop a one-dimensional scattering density profile (SDP) model suitable for the analysis of the experimental data. The joint refinement of such data (i.e. SANS and SAXS) provides the area per lipid that is then used in the fixed-area simulation. In the final step, the

direct comparison of simulated-to-experimental data results in the structure of PG bilayers.

2574-Pos Board B344

A Novel Framework for In-House High throughput Measurements of Lipid Phase Behaviour

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The biomechanical properties of lipid bilayers are known to regulate protein function and activity. These include parameters such as the stored elastic stress, lateral pressure profiles, spontaneous curvature, bending rigidity and charge density. Unfortunately, these parameters have only been characterised for a limited number of lipid systems due to lack of high throughput techniques that can make such measurements. We present a novel high-throughput laboratory based small angle X-ray scattering beamline which is aimed at generating a biologically relevant database of parameters for characterizing lipid behaviour. The system, capable of simultaneously running over 100 samples in 8 different temperature controlled environment at a time is capable of undertaking phase behaviour measurements (between 4^{o}C and 80^{o}C) for lipid assemblies under biologically relevant conditions. The system is fully automated and based around a labview widget interface which brings together camera and sample chamber control. Using this platform and fluctuation mode analysis we have determined the biomechanical properties of binary lipid systems and correlated these with *in-vitro* membrane-protein function. This provides a quantitative framework between membrane composition and structure and membrane protein activity.

2575-Pos Board B345

Shape Entropy and the Time Scales for Thermodynamics in Biological Systems

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Mitochondrial inner membranes show coexistence between tubular and lamellar structures. The lipid molecules in these membranes represent a mobile pool whose observed redistribution on the time scale of seconds ensures that the chemical potentials of the lipid in the tubes and in the lamella be equal. This allows for easy inter-conversion of these shapes and makes possible the entropic stabilization of an ensemble of different shapes. Such shape entropy stabilization on a timescale of seconds accounts for observed morphology.

2576-Pos Board B346

Investigation of Lipid Distribution by Coherent Anti-Stokes Raman Scattering

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The chemical identity of individual lipids in cellular systems is believed to modulate the function of organelles and macro molecular assemblies. It has been shown that lipid composition can alter the rate of membrane protein conformational changes. In particular, variations in saturated and unsaturated lipids are believed to modulate the mechanical properties of the bilayer matrix supporting membrane proteins. Thus far, it has been challenging to identify the specific lipids that interact to modulate intact membranes. To study these phenomena, we have developed a multiplex coherent anti-Stokes microscope to spectroscopically distinguish the chemical composition of lipids in model and cellular systems. CARS imaging is a valuable tool for studying lipids in biological systems. We seek to gain spectroscopic information to discern biochemical gradients and interactions. The microscope utilizes a super continuum stokes beam and a spectrally narrow picosecond probe to measure a selection of vibrational modes simultaneously providing a chemical fingerprint for each pixel. Using this microscope we have detected vibrational bands that can be attributed to specific lipid components, such as the choline head group and are exploring its use in intact cellular systems.

2577-Pos Board B347

Computer Simulation of Cytoskeleton-Induced Blebbing in Lipid Membranes

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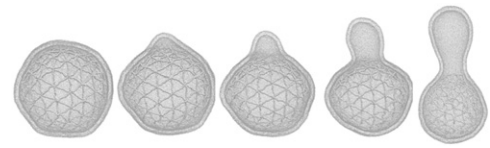
Blebs are balloon-shaped membrane protrusions that form during many physiological processes such as cytokinesis, cell motility and apoptosis. Using computer simulation of a particle-based model for self-assembled lipid bilayers coupled to an elastic meshwork, we investigated the phase behavior and kinetics of blebbing. We found that for small values of the mismatch parameter, defined as the ratio between the area of the lipid bilayer divided by the rest area of the cytoskeleton, the equilibrium state is that of a homogeneous vesicle with the cytoskeleton conforming to the bilayer. However, for large values of a mismatch parameter, the equilibrium state is that of a blebbed vesicle. We also found that blebbing can be induced when the cytoskeleton is subject to a localized ablation or a uniform compression. The obtained results are qualitatively in agreement with the experimental evidence and the model opens up the possibility to study the kinetics of bleb formation in detail. This work is supported by NSF grants (DMR 0812470 and DMR 0755447).



2578-Pos Board B348

Imaging of Supported Bilayers Modified by Butanol and Hexanol, and by the Solvent Decane

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Ion channels, including voltage-gated channels, are often studied in planar bilayer model membranesthat of necessity incorporate a solvent, typically decane. We are interested in the bilayer mechanics of channel function and have examined the electrophysiology of KvAP channels in bilayers modified by short chain alkanols and by cholesterol (Finol-Urdaneta et al. 2010, Biophysical Journal 98:762). Since both alkanols and cholesterol affect KvAP conductance and kinetics, we need a better understanding of how the mechanical status of these ''defined'' bilayers is affected by the presence of decane. We prepare supported bilayers of DPPC, DOPC/DPPC 1:1 and DOPC/egg sphingomyelin/cholesterol 2:2:1 (''DEC221'') by vesicle fusion on mica in the presence and absence of decane, and image them with tapping-mode atomic force microscopy (AFM) in aqueous solution. Decane modifies the domain morphology of DEC221 but has minimal effect on DOPC/DPPC or DPPC bilayer patches. Butanol and hexanol in the absence of decane reduce the percent coverage of the liquid-ordered phase in DEC221 and the gel phase in DOPC/DPPC, and induce an interdigitated phase of lower height in DPPC bilayer patches, consistent with literature results for ethanol. Fluorescence assays in vesicles confirm the alkanol-induced increases in fluidity. This work aims to elucidate the interplay between bilayer lipid molecules and solvent molecules in determining the response of planar bilayers to membrane-perturbing additives.

2579-Pos Board B349

Sucrose Exclusion and Inclusion in a Lipid-Water System

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Sugars are known to strongly affect lipid-water phase behavior and often serve as protectants against cold, heat, and dehydration. However, a detailed picture of how sugars interact with lipids remains an active question. We have determined the phase diagram of the lipid SOPE (1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) as a function of sucrose concentration by means of DSC and laser-light scattering. Using thermodynamic arguments, our data can be used to determine the interfacial concentration of sucrose in lipid-water-sucrose mixtures. Our preliminary results are consistent with a model of sucrose being excluded in a thin layer of water immediately in front of the lipid head groups, but partially included in the gaps between lipid head groups in the fluid lamellar phase and in the core of the inverted hexagonal phase.

2580-Pos Board B350

Membrane Area Deformation under Osmotic Stress: Deuterium NMR Approach

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We address the hypothesis that the sensitivity of lipid bilayers to pressure, temperature, and osmotic stress represents influences of non-specific lipid-protein interactions on functions of cellular membranes [1,2]. Measurements of membrane structural parameters such as bilayer thickness and area per lipid employ a mean-torque analysis [3] of ^{2}H solid-state NMR order parameters (S_{CD}). NMR lipid order parameters are very sensitive to changes in cross-sectional area per molecule. We observed striking ($\approx$20%) changes in structural properties (decrease in area per lipid and increase in bilayer thickness) when $\approx$200 atmospheres of pressure (dehydration pressure or osmotic pressure) are applied to lipid (DMPC) bilayers in the liquid-crystalline state. We show the