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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°C and 80°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.

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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.

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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.

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Computer Simulation of Cytoskeleton-Induced Blebbing in Lipid Membranes

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¹University of Memphis, Memphis, TN, USA, ²IIT Madras, Chennai, India. 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 possi-

bility to study the kinetics of bleb formation in detail. This work is supported by NSF grants (DMR 0812470 and DMR 0755447).



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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.

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Sucrose Exclusion and Inclusion in a Lipid-Water System Kyle T. Bussis, Evan M. Jensen, Paul E. Harper.

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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 lipidwater-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.

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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 ²H 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 ≈ 200 atmospheres of pressure (dehydration pressure or osmotic pressure) are applied to lipid (DMPC) bilayers in the liquid-crystalline state. We show the