

The energetics of membrane proteins and cellular functions are modulated significantly by membrane properties [1]. Accurate knowledge of cross-sectional area/lipid of membrane systems is pertinent for molecular dynamics simulations [2,3]. Here we address the sensitivity of lipid bilayer structure and fluctuations to osmotic pressure and temperature using solid-state ^2H NMR spectroscopy. Applied stress allows us to probe intermembrane interactions including collective membrane motions and lipid protrusions [1,3]. Through ^2H NMR studies [3] we obtain striking evidence for bilayer deformation due to application of osmotic pressure to model membranes (DMPC- d_{54}) using polyethylene glycol as osmolyte. The average structure of the membrane is manifested in the segmental order parameters (S_{CD}) of the lipids that are measured with ^2H NMR spectroscopy. Measurements of membrane structural parameters such as bilayer thickness and area per lipid employ a mean-torque analysis of ^2H NMR order parameters (S_{CD}) [4]. These NMR measurements allow us to interpret the free energy cost of bilayer deformation when combined with complementary X-ray observables. NMR results are used to calculate the osmotic coefficient to distinguish the different regimes of intermolecular forces, thereby yielding insights into bilayer separation forces [2]. We calculated the elastic area compressibility modulus that describes the membrane deformation with osmotic pressure. The present NMR study distinguishes between different intermembrane forces, and suggests that the undulations dominate at intermediate intermembrane distances whereas protrusions act at short distances. The thermodynamic descriptions of these experimental measurements shed light on the effect of osmotic pressure on membranes and their implications for protein functions. [1] M.F. Brown *et al.* (2002) *JACS* **124**,8471-8484. [2] K.J. Mallikarjunaiah *et al.* (2011) *BJ* **100**, 98-107. [3] H.I. Petrache *et al.* (2000) *BJ79*, 3172-3192. [4] K.J. Mallikarjunaiah *et al.* (2012) to be submitted to *PCCP*.

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Ternary Model Membrane System with Thicker Liquid Disordered Phases, Confirmed with Atomic Force Microscopy and Fluorescence Microscopy

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Model lipid membranes containing ternary mixtures of a lipid with a high melting temperature (T_m), a low T_m , and a sterol can phase separate into two coexisting liquid phases over a range of temperatures and compositions. The two phases are termed liquid-ordered (Lo) and liquid-disordered (Ld). In mammalian cell membranes, it is generally assumed that the Lo phase is thicker than the Ld phase. We hypothesized that increasing the chain length of the low- T_m lipid would give a ternary system with a thicker Ld phase. In practice, this is difficult to achieve. Here our high- T_m lipid was DPPC and our long-chained, low- T_m lipid was Di(22:1)PC. We deposited giant phase-separated vesicles made from different compositions of Di(22:1)PC/DPPC/Cholesterol on mica to form supported lipid bilayers. The area fractions of the thicker phase measured by atomic force microscopy agreed with area fractions of the liquid disordered phase measured by fluorescence microscopy. This was true whether the Lo phase or the Ld phase comprised the larger area fraction. To our knowledge, this is the first experimentally determined system in which the thicker liquid phase is not the more ordered phase, showing that height and order are not always directly correlated.

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Effect of Hybrid Lipid on Line Tension and Universality in Lipid Membranes

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Giant unilamellar vesicles (GUVs) can be used for direct investigation of many phenomena. When they are made of at least three components, one being cholesterol, one a high-melting-point lipid and one a low-melting-point lipid, GUVs can exhibit coexisting liquid ordered -liquid disordered phases resulting in micron-sized membrane domains. The energy per unit length of boundary is called line tension, which affects the sizes and shapes of the domains. Line tension depends on the temperature of the system according to power law and vanishes at critical temperature. Universal scaling behavior can help us to understand the phase behavior of many different systems. Systems in the same universality class represent similar collective behavior in phase transitions apart although they have different physical features. Critical exponents characterize the continuous phase transition of systems, and all systems belonging to a universality class will have the same critical exponents. In this work, we measured the critical exponent related to line tension using fluo-

rescence microscopy and image processing. We investigated the effects of hybrid lipid on line tension and critical exponent. Hybrid lipids are abundant in cell membranes. One chain of hybrid lipid is saturated and the other one is unsaturated, because of which they behave as lineactants and can reduce the line tension. We prepared GUVs with three different compositions: DOPC/DSPC/Cholesterol 30:45:25, DOPC/DSPC/POPC/Cholesterol 22.5:45:25:7.5, DOPC/DSPC/POPC/Cholesterol 15:45:55:25. The first system does not contain hybrid lipid; but in second and third systems, 25% and 50% of unsaturated lipid (DOPC) were replaced with hybrid lipid (POPC), respectively. Our results show that the critical exponent associated with line tension gradually increase with hybrid lipid concentration. Having different values of critical exponent in different mixtures indicate that lipid bilayers cannot be classified in a universality class.

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Cholesteryl Phosphocholine forms Fluid Bilayer Membranes with Saturated or Monounsaturated Ceramides

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Some ceramides have been shown to displace cholesterol from liquid ordered domains rich in sphingomyelin or saturated phosphatidylcholine in model membranes. The addition of ceramide will stabilize the formed ceramide/sphingomyelin domains and the subsequent partitioning of cholesterol to the disordered phase will also increase order and stabilization in this phase. The displacement of cholesterol may be due to more favorable interactions of ceramide with the ordered phase lipids, mainly sphingomyelin, compared to cholesterol. The ceramide could also interact less favorably with the disordered phase lipids compared to cholesterol, driving it into the ordered domains. It would also be of interest to better understand how cholesterol and ceramide can interact with each other in bilayer membranes. To study these questions, we have prepared a cholesterol analog with a phosphocholine head group on the 3-oxygen. We have studied the distribution and behavior of chol-PC and ceramides in bilayer systems on their own, and together with saturated and monounsaturated phospholipids. Chol-PC together with ceramide was able to form stable bilayer phases (extruded LUVs, electroformed GUVs). Phosphatidylcholine acyl chain order measurements showed that chol-PC was less efficient in inducing acyl chain order compared to cholesterol. Fluorescence quenching measurements in POPC/ceramide/sterol mixtures further showed a decrease in ordered domain stability of saturated ceramide in the presence of cholesterol or chol-PC. We have also examined the possibility to use chol-PC:ceramide mixtures to load ceramide into cultured cells. Proliferation experiments on HeLa and FRTL-5 cells indicates that chol-PC:ceramide bilayer mixtures are more efficient in inducing apoptosis than DMSO:ceramide mixtures. Our results suggest that chol-PC and ceramide interact with each other under specific conditions, and that the binary bilayers could constitute an interesting formulation for providing cells and tissues with solvent-free ceramides.

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Effects of Polyunsaturated Acyl Chains on Coexisting Liquid Ordered/Liquid Disordered Phases

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Ternary mixtures of POPC, sphingomyelin (SM) and cholesterol (Chol) form coexisting liquid ordered and liquid disordered fluid phases over a wide range of molar ratios. We examined the effects of polyunsaturated acyl chains in this ternary system by incrementally exchanging SDPC (18:0,22:6 PC) for POPC. All measurements were performed on extruded LUVs formed above the main phase transition of the SM. Changes in bilayer properties of the two phases were monitored with NBD linked to di-16:0 PE, which partitions into the liquid ordered phase, and NBD linked to di-18:1 PE which partitions into the liquid disordered phase. Fluorescence lifetime and anisotropy decay dynamics of both fluorescent probes were assessed via frequency-domain measurements collected from 5 to 300 MHz. In both phases the exchange of SDPC for POPC reduced the average fluorescence lifetime of the two probes from the value observed in 1/1/1 POPC/SM/chol. In both phases the lifetime was reduced to a value similar to that observed in a ternary mixture consisting of 8/1/1 POPC/SM/chol. In the liquid disordered phase the addition of SDPC had essentially no influence on probe dynamics, while in the liquid ordered phase SDPC caused a significant acceleration of probe rotational motion.