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 $^2$H NMR spectroscopy. Applied stress allows us to probe intermembrane interactions including collective membrane motions and lipid protrusions [1,3]. Through spectroscopy. Applied stress allows us to probe intermembrane interactions 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) B79, 3172-3192. [4] K.J. Mallikarjunaiah et al. (2012) to be published in PCCP.

3036-Pos Board B191
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 (Tm), a low Tm, and a sterol can phase separate into two coexisting liquid phases over a range of temperatures and compositions. The two phases are termed liquid-disordered (Lo) and liquid-ordered (Ld). In mammalian cell membranes, it is generally assumed that the Lo phase is thinner than the Ld phase. We hypothesized that increasing the chain length of the low-Tm lipid would give a ternary system with a thicker Ld phase. In practice, this is difficult to achieve. Here our high-Tm lipid was DPPC and our long-chained, low-Tm lipid was Dst(22:1)PC using polyoxyethylene glycol as osmolyte. The average structure of the membrane is manifested in the segmental order parameters (S2g) of the lipids that are measured with $^2$H NMR spectroscopy. Measurements of membrane structural parameters such as bilayer thickness and area per lipid employ a mean-torque analysis of $^2$H NMR order parameters (S2g) [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) B79, 3172-3192. [4] K.J. Mallikarjunaiah et al. (2012) to be published in PCCP.

3037-Pos Board B192
Effect of Hybrid Lipid on Line Tension and Unversality 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 micro-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 varies 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 fluorescence 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 inactants 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.

3038-Pos Board B193
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/spinhgomyelin 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 or 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.

3039-Pos Board B194
Effects of Polysaturated 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 polysaturated 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 disordered phase measured by atomic force microscopy for micron 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.
This result was unexpected since it was assumed that the SDPC would be present only in the liquid disordered phase. FRET measurements using di-16:0-PE labeled with NBD and di-18:1 PE labeled with rhodamine were performed in order to monitor changes possible changes in domain size induced by the exchange of POPC for SDPC. Initial results show similar transfer efficiency between these two probes in 1/1 POPC/SM/chol and in 1/2/3/3 POPC/SDPC/SM/chol, suggesting the presence of SDPC does not greatly perturb domain size and structure.

3040-Pos Board B195
Improved Charmm Force Field for Polysaturated Fatty Acid Chains, a Study on DAPC Membranes
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The present research is a part of a collaborative effort to improve the CHARMM force field (FF) parameters, referred to as C36p, for accurate prediction of the properties of polysaturated fatty acid (PUFA) chains in lipid membranes. The focus of our study was to test the accuracy of C36p FF. Molecular dynamics (MD) simulations were used to study the behavior of 1,2-di-racchidonyl-phosphatidylcholine (DAPC), a PUFA with two hydrophobic tails with identical degree of unsaturation. CHARMM and NAMDII software packages were used to equilibrate and analyze the bilayer systems. The system consisted of a DAPC bilayer formed by 72 fully hydrated lipids with periodic boundary conditions. The simulations were performed in the NPT (constant particle number, pressure, and temperature) ensemble at 1 bar and 323.15 K for 100 ns. Simulations with the C36p FF resulted in more accurate membrane properties such as surface area per lipid, deuterium order parameters, electron density profiles, and C-H spin-lattice relaxation times. Efforts continue to improve the FF for accurate prediction on the rigidity of PUFA lipid membranes and orientation of cholesterol within these bilayers.

Endnotes:
1CHARMM(Chemistry at HARvard Macromolecular Mechanics). http://www.charmm.org
2NAMD, Scalable Molecular Dynamics. http://www.ks.uiuc.edu/Research/namd/

3041-Pos Board B196
Interaction of α-Tocopherol with a Polysaturated Lipid Studied by MD Simulations
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Polysaturated phospholipids are essential components of neural membranes and their effect on membrane architecture is proposed to be the molecular origin of a myriad of health benefits. A downside of polysaturated phospholipids is that they are highly susceptible to oxidation due to the presence of multiple double bonds. α-Tocopherol is the most biologically active component in a family of phenolic compounds that comprise vitamin E, which is the major lipid soluble antioxidant in cell membranes. To investigate whether α-tocopheryl preferentially interacts with polysaturated phospholipids for protection against oxidation, we performed MD simulations on 1-stearoyl-2-docosahexaenoyl-phosphatidylcholine (SDPC, 18:0-22:6PC) and 1-stearoyl-2-oleoylphosphatidylcholine (SOPC, 18:0:18:1PC) bilayers containing α-tocopherol. SDPC with a docosahexaenoyl sn-2 chain is polysaturated, while SOPC with an oleoyl sn-2 chain serves as a monounsaturated control. The simulations were run under constant pressure for 200 ns on a system that contained 80 phospholipid molecules, 20 α-tocopherol molecules and 2165 water molecules. We discovered significant differences between the two systems. Notably, α-tocopherol produces a greater increase in order parameters for the stearyl sn-1 chain of SDPC than SOPC, suggesting stronger interaction with the polysaturated phospholipid, and the flip-flop of α-tocopherol across the bilayer is much faster in SDPC than SOPC. We have further quantified the interaction of α-tocopherol with phospholipid by calculating the van der Waals interaction energy between α-tocopherol and the individual lipid chains (sn-1 and sn-2) in SDPC and SOPC. Solid state NMR, neutron scattering and complementary experiments are now underway to test the predictions from the MD simulations.

3042-Pos Board B197
Cholesterol/Phospholipid Bilayer Phase Diagrams from Coarse Grained Simulations
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Coarse grained simulations of membranes containing mixtures of phospholipids and cholesterol at different concentrations and temperatures (below and above the main transition) have been performed. Random mixing without formation of domains was observed. On the contrary, we observed that phase separated fluid systems with different cholesterol concentrations mix into uniform systems in less than 200 nsec. For the gel phase the results are less conclusive due to the two orders of magnitude slower dynamics. The gel to liquid crystalline phase transition is successively weakened by cholesterol while the phase transition temperature increases slightly. The gel phase system undergoes a transition with increasing amounts of cholesterol from a solid ordered phase into a liquid ordered one. In the solid phase, the amplitude of the oscillations in the radial distribution functions decays algebraically with a pre-factor that goes to zero at the two-dimensional solid-liquid transition. The liquid ordered phase is characterized by liquid-like pair correlation functions that decay exponentially to one and have just one detectable peak. Angular correlation functions that measure how the orientation of the lattice vectors in the membrane plane decorrelates with distance were also calculated. They show an algebraic decay with exponent 0.15-0.25 in large regions of the solid ordered phase. This indicates that the liquid ordered phase has more structure than a two-dimensional liquid and may be a hexatic phase.

To explore further whether phase segregation into cholesterol-rich and cholesterol-poor domains is favorable from a free energy point of view, the chemical potential for cholesterol insertion into lipid bilayers at different cholesterol concentrations was calculated from simulations. This shows a small bulk free energy of about 0.3kT per lipid that favors phase separation while a small line tension (a few pN) between cholesterol-rich and -poor regions favors mixing.

3043-Pos Board B198
Molecular View of Phase Coexistence in Model Membranes
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We used computer simulations to investigate phase transformations in lipid monolayers. This is important for understanding lipid-lipid interactions underlaying lateral organization in biological membranes, and the role of phase coexistence in the regulation of surface tension by lung surfactant. Molecular dynamics simulations with the coarse-grained force field MARTINI were employed to achieve large length (~80 nm in lateral dimension) and time (tens of microseconds) scales. Lipid mixtures containing saturated and unsaturated lipids and cholesterol were investigated under varying surface tension and temperature. We reproduced compositional lipid de-mixing and transformation into lipid-condensed (LE) and liquid-condensed (LC) phases, and into liquid-ordered (Lo) and liquid-disordered (Ld) phases. Transformation proceeded via either nucleation and growth, or spinodal decomposition, with distinct coarsening kinetics. Nucleation rate and growth exponents were calculated. Partial lipid areas and phase composition showed a different dependence on surface tension. The domain boundary length increased and the line tension decreased with reducing surface tension. Domains of Lo phase manifested spontaneous curvature at low surface tensions. The surface viscosity of monolayers with phase coexistence increased due to domain reorganization under shear. In the Lo/Ld mixture, strong compositional fluctuations were observed at higher temperatures. Monolayer collapse occurred in the ordered phase (LE or Lo), which then transferred into bilayer and monolayer-bilayer connection. Domains of coexisting phase either increased or reduced monolayer stability. We also investigated lipid bilayers of the same composition. Decreasing surface tension in monolayers and temperature in bilayers had similar effects on the properties of coexisting phases.