can say that the insertion of these synthetic additives lower the temperature of the structural phase transitions comparative to pure lipids and in many cases induce the formation of cubic phases at low temperatures, e.g. 30°C, which corresponds to an increase of the lipid matrix surface curvature. The scattering patterns of the cubic phases are clearly identifiable, despite their intrinsic low resolution. In some cases micellar cubic phases were observed.
In this study we shifted our attention to the influence of small molecules such as urea and TMAO. It is accepted that they have antagonistic effects on the fluidity of lipid membranes. In red blood cells, urea slightly increases the gel-phase domains, but this effect is counteracted by TMAO. We intended to determine how these organic solutes affect the structure of a lipid membrane and determine their contribution to the possible curvature induced on them. We could see a change on the temperature of phase transitions and the formation of induced phases or structures.

**429-Pos Board B209**
**DPPC Monolayers Exhibit an Additional Phase Transition at High Surface Pressure**
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Pulmonary surfactant forms a monolayer at the air/aqueous interface within the lung. During the breath process, the surface pressure (Π) periodically varies from ~40mN/m up to ~70mN/m. The film is mechanically stable during this rapid and reversible expansion.
Pulmonary surfactant consists of ~90% of lipid with 10% integrated proteins. Among its lipid compounds, di-palmitoyl-phosphatidylcholine (DPPC) dominates (~45wt%). DPPC is the only known lipid that can be compressed to very high surface pressure (~70mN/m) before its monolayer collapses. Most probably, this feature contributes to the mechanical stability of the alveoli monolayer. Still, to the best of our knowledge, some details of the compression isotherm presented here and the related structures of the DPPC monolayer were not studied so far.
The liquid-expanded/liquid-condensed phase transition of the DPPC monolayer at ~10mN/m is well known. Here, we report a second phase transition at elevated surface pressure (~50mN/m). The lateral structure of the monolayer at selected states (8mN/m, 20mN/m, 30mN/m, 40mN/m, 50mN/m, 60mN/m, 70mN/m; covering the whole pressure range of the isotherm) was investigated by grazing incidence X-ray diffraction (GIXD). The results report on the 2D packing lattice and on the inter-chain distance dxy. Moreover, the tilt angle of the palmitoyl chains was calculated combining the lattice parameters and the geometrical boundary conditions. The course of the inter-chain distance versus surface pressure exhibits two regimes, separated by the phase transition.

**430-Pos Board B210**
**High Resolution Structure of the Ripple Phase of DMPC Bilayers**
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We have obtained the most detailed ripple phase structure of dimyristoylphosphatidylcholine (DMPC) bilayers by taking synchrotron low and wide angle X-ray scattering (LAXS and WAXS) from highly aligned multilamellar samples. Our LAXS data have 52 measured reflections from which a two-dimensional electron density map was obtained at higher resolution than earlier maps obtained from less than half as many reflections. Consistent with the previous lower resolution study, the bilayer has a ripple amplitude of 18.5 Å with a thicker and longer major arm and a thinner and shorter minor arm, but the features are sharper allowing better estimates for the modulated bilayer profile and the distribution of headgroups along the aqueous interface. WAXS scattering, employing both grazing incidence and transmission geometry, revealed two sharp Bragg rod reflections with small out-of plane $q_z$ components, narrow in-plane $q_r$ width consistent with high lateral order, and a breadth in $q_z$ consistent with tight coupling of the chains from opposite monolayers. Analysis shows that the major arm hydrocarbon chains are tilted in the ripple plane by 18° with respect to the local bilayer normal, contrary to a previous experimental interpretation. Each chain is tilted toward a next nearest neighbor similarly to the gel $L_{\beta F}$ phase rather than the more usual $L_{\beta I}$ phase. There was no clear signature in the WAXS data relating to the minor arm, consistent with those chains being disordered. However, based on the headgroup electron density, we propose that the minor arm is not just like the fluid $L_\alpha$ phase, as often supposed in the literature, because the maximum chain disorder is offset laterally between the upper and lower monolayers. This asynchronous monolayer modulated melting suggests a direction for better theories of the ripple phase.

**431-Pos Board B211**
**Cation Effects on Zwitterionic Lipid Multilayers**
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Lipid multilayers are found inside eukaryotic cells, as for example in the structure of the Golgi apparatus and around neurons forming myelin sheets. In laboratory, lipid multilayers form even in the absence of proteins. This is due to attractive van der Waals (vdW) forces that are strong enough to balance repulsive forces. In the current model of interbilayer interactions there are three contributions to intermembrane repulsion: electrostatics, bilayer shape fluctuations, and hydration. In this study we use small-angle x-ray scattering, dynamic light scattering, and theory to describe how these forces change in the presence of monovalent ions solution, in particular in the case of lithium ions for which the effects are significantly different than for sodium and potassium.

**432-Pos Board B212**
**Atomically Detailed Lipid Bilayer Models for the Interpretation of Scattering Data**
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We present a new atom density profile model (ADP) for extracting lipid bilayer structural characteristics from scattering data. Bilayer models are optimized using a high dimensional non-linear optimization method to si- multaneously fit small angle neutron (SANS) and X-ray (SAXS) scattering data. Final results are determined from a statistical analysis of many op- timized models. This approach yields data which indicates the precision with which the model and target data set determine structural properties. The model and methods are generalizable to more complex systems, such as bilayers with mixed lipid composition, asymmetric leaflets, or embeded transmembrane proteins.

**433-Pos Board B213**
**Van Der Waals Interactions of Lipid Membranes in Highly Polarizable Solutions**
**Ryan Z. Lybarger**, Horia I. Petrache.
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The van der Waals attraction between lipid membranes depends on the polarizability of solute molecules present in the aqueous space between neighboring membranes [1]. It has been shown that zwitterionic molecules (such as common pH buffers) affect van der Waals forces more strongly than monovalent salt ions [2]. Experimentally, changes in van der Waals forces are detected by x-ray scattering measurements of multilamellar lipid vesicles through the sensitivity of lamellar repeat distances to solution polarizabilities. In this respect, a direct determination of solute polarizabilities would lend support to the interpretation of x-ray data. Previously, we used a method to quantify the polarizabilities of zwitterionic pH buffers by combining mass density and index of refraction to calculate a dimensionless solution function, r(c), which gives the polarizability of hydrated solutes as a function of concentration (c) [3]. Here we present a similar analysis of TAPS (a zwitterionic buffer) and adenosine triphosphate (ATP). [1] V. Adrian Parsegian. Van der Waals Forces: A handbook for Biologists Chemists, Engineers, and Physicists. Cambridge University Press, 2006. [2] Megan M. Koerner, Luis A. Palacio, Johnnie W. Wright, Kelly S. Schweitzer, Bruce D. Ray, and Horia I. Petrache. Electrodynamics of lipid membrane interactions in the presence of zwitterionic buffers. Biophys. J., 101:362-369, 2011. [3] Krzysztof Szymanski and Horia I. Petrache. Composite polarizability and the construction of an invariant function of refraction and mass density for solutions. J. Chem. Phys., 134(144701):7, 2011.

**434-Pos Board B214**
**Chelating Agent Induction of Multiphase Coexistence in Lipid Multilayers**
**Michael Weisman**, Merrell A. Johnson, Bruce D. Ray, Horia I. Petrache.
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Previous work on lipid interactions has shown that zwitterionic buffers affect the attractive and repulsive forces between bilayers. The equilibrium spacing between lipid multilayers as measured by small angle x-ray scattering is determined by the balance of van der Waals (vdW), electrostatic and fluctuation forces. We will present a series of small angle x-ray scattering experiments

that explores how chelating agents such as ethylenediaminetetraacetic acid (EDTA) modify the interactions between membrane. EDTA was shown to induce a phase coexistence in multilamellar systems of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) [1]. In this study we show that the effect of EDTA depends on other mobile ions in solution and lipid type. These data require a reevaluation of theoretical models of lipid bilayer interactions. [1] Johnson et al, Langmuir 2014.

**435-Pos Board B215**

**Hydrocarbon Thickness Dictates Cholesterol's Location, Orientation and Motion in a Phospholipid Bilayer**

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The lateral sequestration of lipids with polyunsaturated fatty acid (PUFA) chains into membrane domains depleted of cholesterol has been hypothesized to have an important role in neurological function. This effect has long been attributed to a strong aversion of the disordered polyunsaturated fatty acid (PUFA) chains to the rigid smooth alpha face of cholesterol. Previously, we have performed neutron diffraction studies of deuterated cholesterol incorporated into bilayers composed of diarachidonoyl phosphatidylcholine (di-20:4 PC, DAPC), a lipid with two omega-6 PUFA chains. It was found that cholesterol sequestered at the bilayer midplane, in contrast to its usual upright orientation with the hydroxyl group located near the lipid/water interface. To date it remains unclear whether or not cholesterol's aversion to PUFA chains is a universal property of PUFAs, a behavior unique to omega-6 PUFAs, or the product of membrane disorder.

Using different physical characterization techniques (i.e., neutron diffraction, NMR) and MD simulations, we obtained detailed structural data that rationalize much of the previously inexplicable data regarding cholesterol's behaviour in PUFA bilayers. We are able to demonstrate that cholesterol's mass distribution in the center of a PUFA bilayer is the same for both omega-6 and omega-3 PUFAs. In addition, neutron, 2H NMR and MD data suggest cholesterol's sequestration into the bilayer center may in fact be driven by hydrophobic thickness mismatch, and not necessarily membrane disorder.

**436-Pos Board B216**

**Membrane Domain Interactions by Monte Carlo Type Analysis of Osmotic Stress Data**

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Diverse physiological processes in living systems depend on fundamental interactions of physical origin on the nanoscopic length scale. Of particular interest are forces acting between membrane domains/rafts across the aqueous phase governing their mutual alignment. Besides bare interactions, such as van der Waals attraction or solvation (hydration) forces, also membrane bending fluctuations, which relate to domains' bending rigidities, need to be considered. We have developed a method based on Monte Carlo simulations and global small-angle X-ray scattering analysis, allowing us to scrutinize osmotic stress data of coexisting liquid-ordered (Lo)/ liquid-disordered (Ld) domains for interdomain interactions. We report results for DSPC/DOPC/cholesterol and DPPC/DOPC/cholesterol lipid mixtures and focus in particular on the bending rigidities of Lo/Ld phases. Results are discussed with respect to effects on membrane-mediated partitioning of proteins in different lipid environments, domain line-tension and size-dependent alignment of like-domains.

This work is supported by the Austrian Science Funds FWF, Project No. P24459.

**437-Pos Board B217**

**How do Cholesterol and Saturated Sphingolipids Affect Acyl Chain Order in the Fluid Phase of Binary POPC Bilayers - a Study with 1-oleoyl-2-propionyl-DPH-sn-glycero-3-phosphocholine**

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It is known that cholesterol increases the order in the fluid phase of a lipid bilayer but it has remained unclear whether or not various sphingolipids also have an ordering effect on the bulk phase ordering properties in the fluid phase. The acyl chain order in the gel phases has previously been reported with trans-parinic acid (tPA; steady state anisotropy or lifetime analysis) but acyl chain order in the fluid phase phase separated systems has been mostly measured indirectly, e.g. with 1,6-Diphenyl-1,-3,5-hexatriene (DPH) or N-rhodamine.dipalmitoylphosphatidyletanolamine (Rho-DOPE). By using a 1-oleoyl-2-propionyl-DPH-sn-glycero-3-phosphocholine (18:1-DPH-PC) as a reporter molecule the order in the fluid phase could be measured since the unsaturated probe has preferential partitioning into the disordered phase. The systems we studied were: POPC with 50 mol% palmitoyl sphingomyelin (PSM), POPC with 29 mol% of either palmitoyl ceramide (PCer), palmitoyl galactosyl ceramide (PGalCer) or palmitoyl glucosyl ceramide (PGluCer). In the above bilayer systems (with coexisting disordered and ordered phases present), 18:1-DPH-PC steady state anisotropy reported no gel phase melting during temperature ramps. This contrasts with tPA, whose anisotropy in binary bilayers reported meltings of the ordered phase. 18:1-DPH-PC steady state anisotropy measurements at 23°C showed that PCer, PGlcCer, PGalCer addition to a fluid POPC bilayer had a very minor ordering effect compared to cholesterol addition. These results indicate that cholesterol is superior to saturated sphingolipids to increase order in the fluid phase and that 18:1-DPH-PC is a fluorophore suitable to measure membrane order in the fluid phase.

**438-Pos Board B218**

**Partitioning of the Transmembrane Peptide GWALP23 between Lo and Ld Phases in Macro and Nanoscale Domains. Nanometer-Scale Domains can be Treated as a Phase**

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If liquid-ordered (Lo) and liquid-disordered (Ld) domains coexist in plasma membrane (PM), then the domain size, shape, and morphology can influence membrane behavior. For multi-component lipid mixtures that model the PM, this phase domain morphology can be controlled by lipid composition. Here we find the partition coefficient of a transmembrane peptide (GWALP23) between Lo and Ld phases, for mixtures forming macro domains (bSM or DSPC/DOPC/Chol) or nanoscale domains (bSM or DSPC/POPC/Chol). The WALP family of peptides has proven to be a useful model for investigating the fundamental principles governing protein-lipid interactions. GWALP23 presents 23 amino acid residues with the sequence (GGAF FLALALALALALALWLAGA). By use of Forster Resonance Energy Transfer (FRET), we measured the phase preference of GWALP23, finding favorable partition into Ld phase for all four systems. However, care must be taken when comparing the absolute values of the partition coefficient obtained for macro and nanoscale domains, since the small domain size is a key factor in the measured FRET. In addition, we used the fluorescence emission of 18:1,18:1-LR-PE to measure the partition coefficient of this dye between Lo and Ld phases in the case of macro domains (DSPC/DOPC/Chol) and nanodomains (DSPC/POPC/Chol), finding similar values in each case. Taken together, our results imply that nanodomains can be treated as a phase.

**439-Pos Board B219**

**Cell Cycle Position Determines Critical Temperatures in Plasma Membrane Vesicles**

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Giant plasma membrane vesicles (GPMVs) isolated from RBL-2H3 cells appear uniform at physiological temperatures, contain coexisting liquid-ordered and liquid-disordered phases at low temperatures, and experience micron-sized critical fluctuations close to their critical temperature. Individual vesicles have well-defined critical temperatures yet there is significant vesicle to vesicle variation even when GPMVs are isolated from a plate of seemingly identical cells. In this study, we explore if heterogeneity in critical temperatures arise, at least in part, from cells being at different stages of the cell cycle. Populations of cells were synchronized at S, G2, M, and G1 stages using a double