development process and to avail pharmaceutical company-vetted drug candidates to academicians who wish to explore novel strategies for preventing and treating malaria, the Medicines for Malaria Venture developed the Malaria Box. To assist in this effort, and to reduce the expenses required for drug development, we tested the 80 most potent compounds from the Malaria Box for their bilayer-perturbing effects, as sensed by a membrane-spanning channel, as a proxy for a generic membrane protein. Specifically, we used a gramicidin-based stopped-flow fluorescence assay to probe whether the compounds altered lipid bilayer properties at the concentrations where they are used to inhibit/kill the malaria parasites. Membrane-active compounds are expected to be ubiquitous modifiers of membrane protein conformational changes, meaning that they are predicted to be toxic (above some threshold concentration). Among the compounds tested, four altered membrane properties ($p<0.05$), and one of them MMV007384 was a potent bilayer-perturbing compound that should be used with caution, if at all. This compound was indeed known to be toxic in cell-based screens, suggesting that one can use the gramicidin-based fluorescence to test for cell toxicity.

424-Pos Board B204
Predicting Drug Toxicity: Early Detection of Likely Failures in Drug Development
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It remains a challenge to predict whether or not a new drug candidate will have undesirable side-effects in the clinic. Many biologically active molecules, including drugs and drug-leads, are amphiphiles that partition into lipid bilayers, which may alter bilayer physical properties, thereby modulating membrane protein function. Such bilayer-modifying molecules may be promiscuous modifiers of membrane protein function, raising the possibility that they have off-target effects. Thus, it may be possible to predict whether a compound will have important off-target effects based on quantitative studies on the compound's bilayer-modifying potential. We developed an assay to quantify the bilayer-modifying potential of large numbers of compounds. Using a gramicidin-based fluorescence assay (GBFA), which reports how a compound alters the gramicidin monomer<->dimer equilibrium, we have shown that many drug and drug-leads alter lipid bilayer properties at the concentrations where these compounds become indiscriminate modifiers of membrane protein function. Such indiscriminate modifiers of membrane protein function are likely to have off target effects; we pursued this question in a study on a library of compounds that had been tested for cytotoxicity in "high-content" screening assays that quantified cellular ATP levels, nuclear morphology, nuclear membrane integrity, and apoptosis in immortalized liver, lung, and neuronal cell lines. We tested 134 compounds (40 non-toxic, 40 moderately toxic and 54 highly toxic) using the GBFA (the library was "blinded" until the results of the GBFA were known) and found that the GBFA predicts cellular toxicity, with the Area Under The Curve of the Receiver-Operating Characteristic being 0.84. These results further support a mechanism by which amphiphiles exert their toxicity, namely by altering lipid bilayer physical properties and that such an in vitro measurement could be used as a warning sign for off-target biological effects in drug discovery efforts.

425-Pos Board B205
Understanding the Function of the Cyclic Antifungal Lipopeptide Fengycin using All-Atom Md Simulation
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Fengycin is one of a class of cyclic lipopeptides synthesized by the bacterial genus Bacillus. Many bacteria synthesize similar cyclic peptides, some of which have antifungal or even antibacterial properties, so studying how they interact with membranes is a promising path for drug development. Previously, we ran a series of coarse-grained molecular dynamics simulation studies using MARTINI force field exploring the interactions of fengycin with models for bacterial and fungal membranes. The results suggested that the peptide's ability to aggregate and deform the membrane depended on the nature of the surrounding lipid headgroups, and that these interactions might be the origins of its selectivity. However, coarse-grained models by definition lack atomic-level resolution, so all-atom simulations are needed to confirm and expand on these results. First, we developed parameters for several unusual chemical moieties found in fengycin, such as the cyclization between the C-terminus and a tyrosine side chain, as well as the amide linkage between Glu and β-hydroxy palmitic acid. We validated these parameters via simulations of isolated and clustered fengycin molecules in water, as well as simulations of it bound to two membrane compositions: POPC (to model mammalian or fungal membranes) and 2:1 POPE:POPG (to model bacterial membranes). The analysis of these simulation will reveal more details of the interactions between fengycin and membranes.

Membrane Structure I

426-Pos Board B206
Scattering from Laterally Heterogeneous Vesicles: An Analytical form Factor for Multiple Domains
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It is widely accepted that lateral heterogeneity in the plasma membrane (PM) plays a role in signaling, transport, and the pathogenesis of some viruses and bacteria. In many cases, the size and connectivity of "raft" domains is crucial for understanding their genesis and mode of action, yet detailed knowledge is still lacking. For example, the observation of ~ 10 nm diameter rafts in the PM of unstimulated cells, as well as in multicomponent lipid-only model membranes, has led to several theories explaining nanodomain stability, including microemulsions, critical fluctuations, the presence of line active molecules, or competing interactions (e.g., line tension and bending energy). Unique among biophysical tools, small-angle neutron scattering can in principle give detailed information about the size, shape and spatial arrangement of domains. We present a general theory for scattering from laterally heterogeneous vesicles, including form factors for vesicles containing an arbitrary number of domains. These form factors are then applied to the analysis of neutron scattering data from phase-separated vesicles, with an emphasis on distinguishing the size and spatial arrangement of domains. We discuss important experimental considerations, including the use of contrast matching to highlight different structural features.

427-Pos Board B207
Experimental Assessment of Tilt-Dependent Thermal Fluctuations in Lipid Bilayers
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For length scales shorter than several membrane thicknesses, many molecular dynamics simulations of single component lipid bilayers have reported significant deviations in the measured height-height fluctuation spectrum compared to predictions from the Helfrich free energy. Recently, others have posited membrane models that depend on molecular tilt and that have been shown to be consistent with the aforementioned deviations. We present the first experimental support for a tilt-dependent theory for biomembrane mechanics. X-ray scattering from a liquid crystalline stack of oriented fluid phase lipid bilayers was collected and compared to the predictions of tilt-dependent and tilt-independent membrane models. Both tilt-dependent and -independent models satisfactorily fit the X-ray data dominated by in-plane correlation lengths much greater than membrane thickness (> 100 Å), but only a tilt-dependent model accounts for X-ray data primarily attributable to shorter length correlations. By fitting the measured X-ray scattering intensity, both the bending modulus $K_c = 8.3 \pm 0.6\times10^{-20}$ J and the tilt modulus $K_\theta = 95 \pm 7$ mN/m were determined for DOPC lipid bilayers at 30°C. Our experimental results support the enrichment of the classic Helfrich continuum model to include molecular tilt. Since the size of many biomembrane related molecules is similar to tilt-dependent length scales, the tilt modulus may be highly sensitive to various membrane-biomolecule interactions.

428-Pos Board B208
Structural Effects of Urea and Tmo on Lipid Bilayers
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Lipid bilayers can display a wide range of morphologies and are simple models for the cell membrane, that not only defines the cell limits but also provides a matrix for anchoring a variety of substances, e.g, membrane proteins, glycolipids, etc., that play an essential role in the cell.
Recently, we have been studying the structural effects of synthetic quinones on lipid model membranes, in order to investigate their contribution to morphologies possibly involved in the electron transfer process. Summarizing, we

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_α 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
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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
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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