our group. It was found that both local and general anesthetics display very similar effects on stimulation-response curves of the median nerve. Our findings support the recent thermodynamic theory for nerve pulse generation and propagation (electromechanical solitons, Heimburg & Jackson, PNAS 2005).

1241-Pos Board B133
Why are the Actions of Anesthetics on GABA-A Universal?
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There is a long-standing debate on whether general anesthetics act through a non-specific perturbation of bilayer physical properties or through binding to specific sites within ion channels, particularly the GABA-A receptor. In this study, we demonstrate that a series of liquid n-alcohol general anesthetics lower phase transition temperatures in giant plasma membrane vesicles, which have previously been shown to sit close to a miscibility critical point. All n-alcohols depress critical temperatures (Tc) by $4 \pm 1^{\circ}C$ when added to vesicles at their anesthetic dose. Current work is investigating if transition temperatures are also depressed when n-alcohols are added to synthetic vesicles with critical lipid compositions. We also performed simulations of simplified receptors embedded in a nearly-critical membrane. In this model, receptor channels can be in two distinct internal states (conducting or non-conducting), and the occupancy of these states is allosterically regulated by their local lipid environment as well as the availability of ligand. We show that model channels with dimensions comparable to that of GABA-A could have their conductance increased by 50% when Tc is lowered by $4^{\circ}C$ in the limit of low ligand concentration. This is in good agreement with experimental observations of GABA-A channels in the presence of general anesthetics (compare to Figure 2b in [1]). Taken together, these findings suggest that general anesthetics can have dramatic effects on the internal states of membrane bound proteins without requiring that they directly bind to specific sites. Instead, we propose that anesthetics may act by lowering the critical temperature of the membrane which in turn allosterically regulates ion channel function.
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1242-Pos Board B134
Small Molecule Interaction with Lipid Bilayers: A Molecular Dynamics Study of Chlorhexidine
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Chlorhexidine is a chemical antiseptic shown to be effective against a wide range of bacteria. It is therefore used in a wide range of industries in products such as: surgical hand washes, mouthwash, industrial sterilization and many other similar applications. It acts specifically against the plasma membrane, causing leakage leading to cell death. Chlorhexidine presents an interesting modelling challenge with a hydrophobic hexane connecting two biguanides (arginine analogues) and two aromatic rings. We conducted molecular dynamic simulations to reproduce the experimental environment of chlorhexidine in a 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer to produce atomic-level information. We constructed an all-atom force field of chlorhexidine from the CHARMM36 force field using well established parameters of certain amino acids. Partial charges were treated differently, which were calculated using GAUSSIAN software. Using GROMACS simulation software we were able to determined that chlorhexidine resides inside of the membrane around the headgroup region of the lipids in a wedge shape. This concurs with previous studies done by this lab using neutron diffraction which have determined that chlorhexidine was located at the membrane aqueous interface.

1243-Pos Board B135
Role of Lipid Composition in Gas Permeation across Biological Membranes
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Gas molecules dissolved in an aqueous environment are pre-dominatly in their hydrophilic charged state because of their respective acid/base equilibria. Overton's rule suggests that permeation of small charged molecules across the membrane boundary is highly restricted and must occur either in their neutral form or through a dedicated transport mechanism. There has been experimental evidence to the effect that the water channel family, Aquaporins, may be responsible for the permeation of neutral gas molecules such as CO2, NH3 , and O2 across biological membranes. This is in apparent contrast to the observation that the permeation rate of neutral gas molecules across pure lipid bilayers is mostly unhindered. It is hence of interest to test the hypothesis the lipid composition of biological membranes may result in a vastly different in gas permeability as composed to pure model membranes.
The lipid composition of physiological membranes is highly complex and further involves asymmetry in the two leaflets. Also, a major component of the cellular lipid bilayers involved in gas permeation, such as those surrounding Red Blood Cells (RBCs), is cholesterol. Cholesterol is known to have a strong clustering effect that increases the packing of the lipids in the membrane. It is possible to study the role of lipid composition and asymmetry through molecular dynamics (MD) simulations by reconstructing atomistically the important characteristics of physiological membranes. Here we present preliminary results of the permeability of model physiological membranes based on atomistic MD simulations.

1244-Pos Board B136
Cardiolipin's Impact on Model Membranes Thermodynamic: Drug-Lipid Interactions and Protein Conformation Implication
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Steady-state fluorescence anisotropy and dynamic light scattering (DLS) were used to determine the thermotropic properties of lipid model systems of bacterial membranes. Diferent lipid proportions of PE:PG:CL were used in order to mimic *Yersinia kristensenii*, *Proteus mirabilis* and *Escherichia Coli* membranes. Cardiolipin's inclusion as a third lipid component of any PE:PG mixture considerably changes the system's properties. The results obtained by these two techniques were shown to be reproducible and were the same within experimental errors, suggesting that either technique can be used to obtain accurate values to characterize the thermotropic behavior of such systems. Moreover they show that the main transition temperatures obtained are undoubtedly cardiolipin dependent. Additionally AFM experiments were performed and these results show that even at small concentration cardiolipin produces important changes not only in the membrane thermotropic properties, but also in the bilayer structure. Moxifloxacin and enrofloxacin's interaction studies with these model systems were also performed and show that cardiolipin's absence greatly influence the conclusions obtained. Preliminary circular dichroism results of an *E. coli* membrane protein, OmpF, reconstituted in different model system membranes, with and without cardiolipin, also point out for its influence on protein's conformation.
Taken all together these results show that cardiolipin's incorporation in model system membranes can have a significant impact on the membrane's properties and its inclusion should be considered when the aim is to construct model system of bacterial membranes.

1245-Pos Board B137
Stressing Lipid Membranes: Effects of Polymers on Membrane Structural Integrity
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Cell membrane dysfunction due to loss of structure integrity is the pathology of tissue death in trauma, muscular dystrophies, reperfusion injuries and some common diseases. It is now established that certain poly(ethylene oxide) (PEO)-based biocompatible polymers, such as Poloxamer 188, Poloxamine 1107, and PEO homopolymers, are effective in sealing of injured cell membranes, and thus prevent acute necrosis. Despite the highly potential application of PEO-based polymers for these medical problems, the fundamental mechanism of how these polymers interact with cell membranes are still under debate. Here, the effects of these polymers on structural integrity of lipid vesicles were explored under osmotic and oxidative stress. Through fluorescence leakage assays, time-lapse fluorescence microscopy, dynamic light scattering and isothermal titration calorimetry, we identified that the surface-adsorbed hydrophilic polymers efficiently inhibits the loss of structural integrity of lipid vesicles under external stimuli, while the insertion of the hydrophobic polymers increases membrane permeability. To elucidate the mechanism by which hydrophilic polymers help restore membrane integrity while their hydrophobic counterparts disrupt it, 1H Overhauser Dynamic Nuclear Polarization (ODNP)-NMR spectroscopy, a newly developed NMR technique that is highly effective in differentiating weak surface adsorption versus translocation of polymers to membranes, was employed to detect polymer-lipid membrane interactions through the modulation of local hydration dynamics in lipid membranes. Our study shows that P188, the most hydrophilic poloxamer known as a membrane sealant, weakly adsorbs onto the membrane surface, yet effectively retards

membrane hydration dynamics. Contrarily, P181, the most hydrophobic poloxamer known as a membrane permeabilizer, initially penetrates past lipid headgroups and enhances intrabilayer water diffusivity. Consequently, our results illustrate that the relative hydrophilic/hydrophobic ratio of the polymer dictates its functions.

1246-Pos Board B138

Lipid-Polymer Membranes as Carrier for L-Tryptophan: Molecular and Metabolic Properties

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A study of the lipopolymers that encapsulate L-tryptophan was carried out with the main goal of obtaining and characterizing vehicles that could be used as drug delivery systems for the treatment of several metabolic diseases that need an incremented systemic L-tryptophan concentration.

Polymeric liposomes were obtained by UV irradiation of vesicles containing 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (DC8,9PC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in 1:1 molar ratio. These polymeric liposomes were also obtained in presence of 10 and 50 mol % of L-tryptophan (respect to total lipid concentration).

Polymerization efficiency in presence of the two mentioned L-tryptophan concentration were studied spectrofotometrically; along with bilayer packing at the polar head region with the probe Merocyanine 540 (MC540). Interaction between lipid-polymer membranes and L-tryptophan was followed by FTIR. Results showed that high L-tryptophan concentration induce the formation of lipopolymers with higher polymeric units, leaded by the higher lipid rigidity adopted in presence of high amino acid concentration. This is a derived implication of the L-tryptophan preferential position interacting at the amine terminal of the choline group.

Stability of lipopolymers with different amounts of L-tryptophan was also studied through release profiles. L-tryptophan release was induced by a concentration gradient and amino acid concentration was determined spectrofotometrically. Polymeric liposomes were able to retain around 80 % of the L-tryptophan after 24 hour. Then, polymeric liposomes with 10 mol % of the amino acid release 5 % more. Nonetheless, retention was high in the elapsed time analysed.

Metabolic activity of the Caco-2 cell line was also studied in the presence of polymeric liposomes with both L-tryptophan concentrations. Cytotoxic effects were low.

In resume, polymeric liposomes studied in this work could be applied as drug delivery systems in order to improve L-tryptophan pharmacodynamics.

1247-Pos Board B139

Controlled Cyclic Measurements of Pulmonary Surfactants at Low Surface Tensions

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Pulmonary surfactants cover the alveoli of the lungs and have a vital function in making the process of breathing easy. During inhalation, the surfactant reduces the surface tension of tissue by a factor of about 15. During exhalation, the surface area of the alveoli decreases making the surfactant even more concentrated on the surface. It is known that the highly ordered solid phase of dipalmitoylphosphatidylcholine (DPPC) sustains the near-zero surface tension on the alveoli during exhalation. In order to model the actual surfactant behavior in the alveoli, measurements at near-zero surface tensions are needed.

We have shown before that the compression speed in a Langmuir trough has a distinct effect on the layer formation of DPPC at low surface tensions and temperatures ranging from 20 °C to 37 °C. In this study we further expand this observation by showing controlled cyclic measurements of DPPC at low surface tensions. The measurements were done on ultrapure water surface at temperatures of 20 °C, 30 °C and 37 °C using a Langmuir trough equipped with a ribbon barrier to prevent monolayer leakage. The measurements show reliable compression measurements of natural phospholipid surfactants at surface tensions down to 15 mN/m and demonstrate the usability of the ribbon barrier method. The measurements can be further expanded to examine the phase transitions and the selective enrichment process of DPPC on the alveoli surface.

1248-Pos Board B140

Directionality of the Nano-Scale Reversible Collapsed Structures in the Pulmonary Surfactant Film

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Up to now, the determination of the orientation of nano-scaled three-dimensional collapse structures formed at the air-water interface within a compressed Langmuir film was not possible by existing experimental techniques. This is however of special importance if pulmonary surfactant films are investigated, which form reversible surface-associated reservoirs or collapse structures at the air-water interface under dynamic lateral compression and expansion. This surfactant efficient mechanism of collapse has been proposed for a mechanically efficient functioning of the pulmonary surfactant lining, present at the air-alveolar interface. The direction of these reservoirs with respect to the interface, however, has remained dicey since decades. To address this question, we have designed a novel methodological approach to perform bidirectional surface imaging of the monomolecular membrane harboring nano-scale collapsed structures. This approach has been applied to investigate the collapsed structures formed in a functional analog of the pulmonary surfactant. Here, we prove that the surface-associated structures of the pulmonary surfactant film form towards the air-phase in contrast to the up to now commonly accepted view of an orientation towards the aqueous-phase.

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1249-Pos Board B141

Membrane Remodelling and Protein Interactions - A Free Energy Perspective

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We investigate the interplay between cell membrane curvature induced at the atomic scale, due to specialized peripheral membrane proteins, and the resulting membrane morphologies, of varying complexity, observed at the meso-scale. The biological membrane, in our approach, is represented by a dynamically triangulated surface while the proteins are modeled as curvature fields on the membrane, that are either isotropic or anisotropic. In order to compare with experiments, we have focused on the ENTH domain containing EPSIN whose curvature field is modeled as isotropic, and on the BAR, Exo70 and ESCRT family proteins whose curvature fields are determined to be anisotropic, both in experiments and in molecular simulations. Thermal undulations in the membrane and cooperativity in the curvature fields, due to the stabilization of a nematic phase, collectively drive the membrane into different morphological states (buds, tubules, etc.) that resemble those in cellular experiments in vivo and vesicle experiments in vitro. The relative stabilities of these self-organized shapes are examined by two approaches to compute the free energy of the system: (i) the Widom insertion technique to compute excess chemical potentials and (ii) thermodynamic integration using the Kirkwood coupling parameter to compute free energies. Building on these methods, we propose a hybrid scheme that couples both the approaches for computing free energies in membrane systems with heterogeneous and phase-segregated protein field - examples being the endoplasmic reticulum (ER) membrane discs with α- calreticulon protein confined to the rim and the vesicular bud formed due to the constriction by ESCRT proteins at its neck.

1250-Pos Board B142

Quantification of Curvature Gradients in Highly Curved Tubular Lipid Bilayers

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Coupling between membrane shape and composition plays an important role in dynamic life of cellular membranes. It becomes increasingly understood that intrinsic curvature preferences of membrane components, proteins and lipids, provides one of the driving forces for such coupling. However, monitoring curvature-driven sorting of membrane components at physiologically relevant time- and length- scales is a challenging task. Here we propose a new approach for real-time quantification of dynamic gradients of membrane curvature and the associated redistribution of membrane component at nanoscale. This method consists in simultaneous measurements of the electrical conductance and fluorescence of the lumen of lipid nanotubes (NTs). By relating changes in the integral conductance of the NT lumen with those in the axial profile of the fluorescence intensity we obtain the geometrical parameters of the nanotube with 10s of nm precision. Furthermore, by varying the electrical potential applied to the NT membrane we can measure, in real time, changes in the elastic moduli of the NT membrane, e.g. upon adsorption of proteins. For basic lipid compositions, the effective bending rigidity measured here coincides with the published values, while entropy-related correction is evident at high curvature stress.