a representative exchangeable apolipoprotein, apoLpIII from Locusta Migratoria, using surface-sensitive X-ray techniques. This model exchangeable apolipoprotein contains a 5-helix bundle and shows high structural homology to the 4-helix bundle in the N-terminus of apoE, a major mammalian exchangeable apolipoprotein. We found that the structure of two diacylglycerols, the proposed binding partner of apoLpIII, dioleoylglycerol (DOG) and 1-palmitoyl, 2-oleoylglycerol (POG), showed great resemblance to other (phospho)lipid monolayers except for details due to differences in the headgroup region. Despite their near identical chemical structures small differences in organization as monolayers were observed between DOG and POG. For the pure apoLpIII monolayer we observed that the unfolded protein was best represented by two distinct regions. This surprising result may originate from the high degree of glycosylation of apoLpIII. The interaction of apoLpIII underneath a densely packed diacylglycerol monolayer causes the surface pressure to increase rapidly. The analysis of the X-ray reflectivity of the lipid/protein system, right after the injection of apoLpIII, shows a diffuse layer underneath the lipid monolayer due to the binding and unfolding of apoLpIII. This may be the first direct visualization of the dynamic unfolding of an exchangeable apolipoprotein at a lipid interface. Our data suggest that the initial interaction occurs when the protein is tilted with respect to the lipid monolayer, a process that would favor the opening of the helix bundle.

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Folding Of Lipid Monolayers Containing Lung Surfactant Proteins SP-B1-25 and SP-C Studied via Coarse-grained Molecular Dynamics Simulations

Susan L. Duncan, Ronald G. Larson.
University of Michigan, Ann Arbor, MI, USA.

To explore the role of lung surfactant proteins SP-B and SP-C in storing and redelivering lipid from lipid monolayers during the compression and re-expansion occurring in lungs during breathing, we simulate the folding of lipid monolayers with and without these proteins. We utilize the recently developed MARTINI coarse-grained force field to simulate monolayers containing pure dipalmitoylphosphatidylcholine (DPPC) and DPPC mixed with palmitoyloleoylphosphatidylglycerol (POPG), palmitic acid (PA), and/or peptides. The peptides considered include the 25-residue N-terminal fragment of SP-B (SP-B$_{1\text{-}25}$), SP-C, and several SP-B$_{1\text{-}25}$ mutants in which charged and hydrophilic residues are replaced by hydrophobic ones, or vice versa. Most of these peptides facilitate folding of the monolayer during compression by a ‘‘zipper’’ mechanism, which is dependent on the formation of a peptide aggregate. However, we find that if the number of hydrophobic residues is decreased significantly monolayer folding does not occur via the ‘‘zipper’’ mechanism. During the re-expansion of folds formed via the ‘‘zipper’’ mechanism, the folds are observed to unfold with the lipids re-entering the monolayer before the peptide aggregates. Our results show several key trends. The addition of POPG to the DPPC monolayer has a fluidizing effect, which assists monolayer folding. In contrast, the addition of PA has a condensing affect. The addition of peptides fluidizes the monolayer and accelerates the folding processes. If the peptides are allowed to aggregate, the peptide aggregate nucleates a defect in the monolayer, further assisting the folding process. The results also show a clear system-size dependence that affects the folding mechanism observed. If the system size is large enough peptide containing monolayers can fold without the formation of a peptide aggregate.

Membrane Structure II

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Structural Changes in DMPG upon changes of ionic strength and pH - What to learn from SANS, DSC, FCS, Flourescence Microscopy, FTIR and Viscosity Measurements

Julia Preu[1], Thomas Gutberlet[2], Thomas Heimburg[1].
[1]Niels Bohr Institue, Copenhagen, Denmark, [2]Juelich Center for Neutron Science, c/o TU Munich, Garching, Germany.

Commonly pure phospholipid membranes are used as models for the more complex real biological membranes. Self-assembling the phospholipids can exhibit a number of different lamellar and nonlamellar phases. They are undergoing a cooperative melting reaction, which is linked to loss in conformational order of the lipid alkyl chains. In certain cases, like for aqueous dispersions of dimyristoyl phosphatidylglycerol (DMPG) a negatively charged phospholipid, this is resulting in an extended network system. The transitions associated are depending on temperature, pressure, lipid concentration and sample environment, such as ionic strength and the pH value (Schneider, M. F. et al., PNAS, 96 (1999) 14312; M.T. Lamy-Freund, K.A. Riske, Chem. Phys. Lipids, 122 (2003) 19).

We studied DMPG dispersions under different pH and ionic strength conditions using methods gaining complementary information about the changes in material properties. In particular we studied the structural changes using small angle neutron scattering (SANS) as well as fluorescence microscopy and the thermodynamical by differential scanning calorimetry (DSC). In addition rheology, Fluorescence Correlation Spectoscopy (FCS) and Fourier-Transform Infrared Spectroscopy (FTIR) was performed. This results in a comprehensive model, taking into account the thermodynamic and structural changes below, in and above the region of the phase transition.

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Formation of Block Liposomes is a General Phenomenon of Charged Membranes

Alexandra Zidovska[1], Kai K. Ewert[1], Joel Quispe[2], Bridget Carragher[2], Clinton S. Potter[2], Cyrus R. Safinya[1].
[1]University of California, Santa Barbara, Santa Barbara, CA, USA,
[2]The Scripps Research Institute, La Jolla, CA, USA.

We have recently reported on the discovery of block liposomes (BLs), a new class of chain-melted (liquid) vesicles, formed in mixtures of curvature-stabilizing hexadecavalent cationic lipid MVLBG2, DOPC and water (Zidovska et al., *Langmuir*, 2008). BLs consist of distinctly shaped, yet connected spheres, pears, tubes, or rods. Unlike typical liposome systems, where spherical vesicles, tubular vesicles, and cylindrical micelles are separated on the macroscopic scale, within a BL, shapes are separated on the nanometer scale. We carried out structural studies of BLs with differential-contrast-microscopy (DIC) and cryo-transmission-electron-microscopy (cryo-TEM) and identified membrane charge and spontaneous membrane curvature as key parameters controlling the BL-formation. BL-formation was believed to be a special capacity of MVLBG2, a newly synthesized highly charged (16+) lipid (Ewert et al., *JACS,* 2006) with giant dendrimer-like headgroup leading to a conical molecular shape resulting into high spontaneous membrane curvature, when incorporated into lipid bilayer. In this work we report formation of BLs for other cationic lipids, demonstrating that BL-formation is a general phenomenon of all charged membranes. We carried out systematic study of binary lipid mixtures comprised of DOPC and a cationic lipid, varying the headgroup size and charge of cationic lipid from 1+, 3+ to 5+. We find that all cationic lipids form BLs on the micrometer and nanometer scale. We have also found that pure DOPC forms BLs in presence of monovalent salt, which is known to cause zwitterionic DOPC to become negatively charged. This latter finding confirms that BL-formation is a general capacity of charged membranes independent of the charge nature. Block liposomes may find a range of applications in chemical and nucleic acid delivery and as building blocks in the design of templates for hierarchical structures. Funding by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347.

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Lipid Bilayer Pre-Transition as the Beginning of the Melting Process: a Periodic Melting

Karin Riske[1], Rafael P. Barroso[2], Cíntia C. Vequi-Suplicy[2], Renato Germano[2], **Vera B. Henriques**[2], M. Teresa Lamy[2].
[1]Departamento de Biofísica, Universidade Federal de São Paulo, São Paulo, Brazil, [2]Instituto de Física - University of São Paulo, São Paulo, Brazil.

We investigate the bilayer pre-ransition, which some lipids present at temperatures below their main phase transition, and which is generally associated to the formation of periodic ripples. Experimentally, we focus on the ubiquitous dipalmytoylphosphatidylcholine (DPPC) and on its charged analog dipalmytoylphosphatidylglycerol (DPPG) at different ionic strengths. Analysis of the excess heat capacity of DPPC and DPPG shows that both pre- and main transitions are part of the melting process. The cooperativeness of DPPG is lower at low ionic strength. Electron spin resonance of spin labels located at the bilayer center reveals the coexistence of gel and fluid domains at temperatures between the pre- and main transitions. Excitation generalized polarization of Laurdan also suggests microphase coexistence in the ripple phase of both lipids. To broaden the knowledge on the ripple phase, we introduce a new statistical model where a next-nearest-neighbor competing interaction is added to the usual two-state model. For the first time, modulated phases, with ordered and disordered lipids naturally appearing in a periodic fashion, are obtained between the homogeneous gel and fluid phases. A better understanding of the different interactions among lipids in a bilayer is of fundamental importance to the full knowledge of the biophysics of natural membranes.

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Phase Transitions In Charged-lipid Membranes: A Statistical Model

Renato G. Nunes[1], C.R. Barbetta[1], M.T. Lamy[1], M.N. Tamashiro[2], V.B. Henriques[1].
[1]Universidade de São Paulo, São Paulo, Brazil, [2]Universidade Estadual de Campinas, São Paulo, Brazil.

Experimental studies of anionic membranes have generated several new and interesting information on their behavior under different pH, salt concentration and temperature conditions. However, good microscopic models is still lacking. We propose a generalization of the statistical model for neutral lipids to include the effect of the ionic charges. The ionization state of the lipid polar headgroup depends on the salt concentration, so one may have either dissociated (charged) or associated (neutral) gel/fluid phases, as well as semi-dissociated phases. The model order parameter, specific heat and conductivity are compared with experimental data of an aqueous dispersion of the phospholipid DMPG (dimyristoyl phosphatidylglycerol).

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Biophysical Characterization Of Phosphatidyl Alcohols In Model Membranes- Effects Of Headgroup Size

Shishir Jaikishan, Anders Björkbom, J. Peter Slotte.
Åbo Akademi University, Turku, Finland.

The polymorphic phase behavior of different phospholipids depends strongly on fine details of their head group and backbone structures. Studies of phospholipid head group modification offers an attractive strategy in elucidating how structural elements of the head group effect membrane properties. We are investigating phosphatidyl alcohols with saturated fatty acyl chains and their interactions with phospholipids and sterols. Molecules of interest are dipalmitoyl glycerophospholipids with methanol, ethanol, propanol and butanol as different headgroups. These molecules can be formed from e.g., corresponding phosphatidylcholines due to the transphosphatidylation activity of phospholipase D. Phosphatidyl ethanol is of physiological relevance since it is increasingly formed in cells of chronic alcoholics. Thermodynamic properties such as phase transition temperatures, heat capacity of transitions and transition cooperativity have been studied with high sensitivity differential scanning calorimetry (DSC). The miscibility and effects of cholesterol on mixtures of these negatively charged phospholipids with high Tm and low Tm phospholipids have been determined using DSC, and with fluorescence spectroscopy using domain-selective lipid probes. The relative acyl-chain order in the bilayer membranes has been assessed using steady-state DPH anisotropy. We will report on the phase behavior of these molecules in simple and more complex bilayer systems. The formation, by the dipalmitoyl phosphatidyl alcohols, of sterol-enriched or ordered domains in fluid bilayer membranes will also be determined. Comparison of the properties of these phosphatidyl alcohols with molecules having closely related structures will help in understanding the relationships between the chemical structures, physical properties and functional properties of the phospholipid molecules in model as well as biological membranes.

2367-Pos Board B337

Measurements of Electrostatic Interactions between Charged Membranes

Matthew J. Justice, Carina M. Poltera, Horia I. Petrache.
Indiana University Purdue University Indianapolis, Indianapolis, IN, USA.

Neighboring lipid membranes experience attractive and repulsive forces. The attractive (van der Waals) force is due to induced change fluctuations and it acts even between neutral membranes. Repulsive forces arise from the tendency of lipids to stay hydrated and can include electrostatic interactions due to accumulation of membrane surface charge. When the attractive vdW force is balanced by repulsive forces, an equilibrium spacing between lamellae is established, as measured by X-ray scattering. This is often the case for neutral membranes. By contrast, lipid bilayers made entirely of charged lipid species such as the negatively charge phosphatidylserine (PS) swell indefinitely (‘‘unbind’’) in the absence of salt indicating the dominance of electrostatic repulsion. To measure the strength of electrostatic interactions in solution, we manipulate membrane charge in two simple ways: by adsorbing ‘‘sticky’’ ions such as bromide onto neutral phosphatidylcholine lipids, and by mixing-in charged lipid species. We will present results and discuss in what conditions membrane electrostatics can lead to unbinding.

2368-Pos Board B338

The Role of Sedimentation and Osmotic Stress in the Regulation of Surface Drag for GUVs Moving in an Electric Field

Ivan A. Rey-Suarez, Guillaume Gay, Alexander Ladino, Esteban Duran,
Alba G. Avila, Juan C. Briceño, Andres Gonzalez-Mancera, Chad Leidy.
Universidad de los Andes, Bogotá, Colombia.

Giant unilamellar vesicles (GUVs) suffer variations in shape when exposed to changes in osmotic stress. These shape variations should alter their drag properties when moving close to a surface by inducing a change in the lubrication drag between the surface and the vesicle due to an excess surface area. Using POPC vesicles labeled with NBD-PE we study the influence that osmotic pressure and sedimentation conditions produce on the dynamics of vesicles moving under the influence of a lateral electric field. As a result we find that vesicles in the 5 - 15 μm size range reach a higher terminal velocity in hypotonic compared to hypertonic conditions. This difference decreases for larger diameters. Confocal fluorescence microscopy is used to measure the shape of the vesicles for different osmotic conditions in order to relate the deformability of the vesicle to the terminal velocities. Additionally, we obtain preliminary measurements on the thickness of the water layer that separates the vesicles from the glass surface, which determines the lubricating drag force. For GUVs that remain mobile this distance ranges between 0.5-3 μm. Variations in the drag conditions are studied using finite element simulations through a 3-D boundary integral method which models the approach of the vesicle to the glass surface. Through this method we study the vesicle deformability, and the limiting value of the water thickness. We relate these results to the movement of the vesicles due to an applied constant force. The theoretical and experimental results are compared using the dimensionless Bond and capillary numbers.

2369-Pos Board B339

Interaction of Buffers with Lipid Membranes

Megan M. Koerner, Ahmad Mossa-Basha, Prashant Srinivasan,
Horia I. Petrache.
Indiana University Purdue University Indianapolis, Indianapolis, IN, USA.

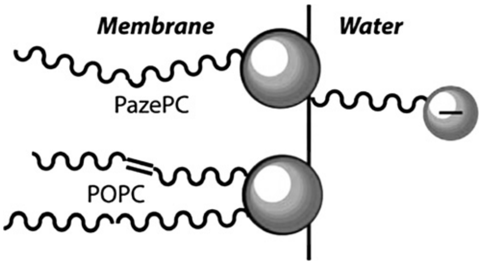
Common buffers including Tris, MES, and MOPS are used to control the pH values of aqueous solutions. The pH, in turn, determines the charged (protonation) state of macromolecules such as proteins, lipids, and DNA. We have investigated the effect of common buffers on the charged state on lipid aggregates. Immersed in water, common lipids often form multilamellar structures. The extremely uniform interlamellar spacings (measurable by X-ray scattering) depend critically on the chemical nature (composition) of the solvent, including the presence of buffers. Multilamellar structures shrink or expand (swell) depending on how interlamellar forces are modified. We present measurements on the modification of lipid interactions by buffers and discuss specific buffer effects that go beyond the non-specific effect of pH.

2370-Pos Board B340

Lipid Gymnastics: Complete Acyl Chain Reversal in Oxidized Phospholipids: Evidence from Molecular Simulations

Himanshu Khandelia, Ole G. Mouritsen.
University of Southern Denmark, Odense, Denmark.

Biological membranes in oxidative environments can contain oxidized phospholipids (OXPLs) with short, polar acyl chains. Two lipid oxidation products of POPC are PoxnoPC and PazePC, which have a carbonyl and an anionic carboxyl group pendant at the end of the short oxidized acyl chain respectively. We have used Molecular Dynamics simulations to investigate the possibility of chain reversal in OXPLs in POPC-OXPL mixtures. The polar azelaoyl chain of PazePC undergoes complete chain reversal without compromising the lipid bilayer integrity at concentrations up to 25% OXPL. The carboxyl group points into the aqueous phase. The carbonyl-containing chain of PoxnoPC is oriented perpendicular to the bilayer normal; and perturbed the lipid packing arrangement more strongly. This work provides molecular evidence of the ‘‘extended lipid conformation’’ in phospholipid membranes. OXPLs are implicated in diseases including cancer and heart disease. The simulations present a clear structural model, using which it will be possible to investigate the interactions of OXPLs with various membrane hosts, ranging from single ions to large proteins. This will ultimately lead to a better understanding of how OXPLs participate in the genesis of diseased states.



2371-Pos Board B341

Headgroup Conformations of Phospholipids from Molecular Dynamics Simulation: Sampling Challenges and Comparison to Experiment

Alexander Vogel[1], Michael F. Brown[2], **Scott Feller[3]**.
[1]University of Halle, Halle, Germany, [2]University of Arizona, Tucson, AZ, USA, [3]Wabash College, Crawfordsville, IN, USA.