825-Pos Board B704
Entropy Driven Like Charched Condensation

Uri Raviv.

The Hebrew Univeristy of Jerusalem, Jerusalem, Israel.

Like charged systems are expected to have repulsive interactions. However, attraction has been observed in a variety of systems, in the presence of multivalent counterions. This includes DNA condensation, as found within virus capsids or chromosomes and the attraction between similarly charged biopolymers such as microtubule or F-actin. Each system exhibits different behaviour and the observations cannot reside within a consistent framework.

We studied the interaction between mixtures of neutral and negatively charged lipids in pure water and in a variety of salt solutions. This is a good model system to study like-charge attraction because the membrane charge density can be controlled by the neutral/charged lipid stoichiometry. The strength of vdW interaction between the membranes, the elastic properties of the membrane and its dimensions can be controlled by the lipid tail structure.

In the meeting results showing how entropy derives long-ranged condensation of liked-charged membranes when no salt is added and under various conditions will be presented. The critical conditions needed for membrane condensation will be discussed.

826-Pos Board B705
Free Energy and Conformation of Hydrocarbons in Lipid Bilayers

Eoin P. Coll, D. Peter Tieleman.

University of Calgary, Calgary, AB, Canada.

Molecular dynamics simulations combined with umbrella sampling allows probing the free energy landscape for small molecules within a lipid bilayer. We have applied this method to several hydrocarbon molecules, some of which are biologically relevant substrates of membrane transporters. Molecules of hexane, decane, hexadecane, and nonacosane have been tested in bilayers of DOPC, and decane and hexadecane in DPPC. It has been found that these hydrophobic molecules overwhelmingly favor localization to the center of the bilayer, and that there is a significant cost to remove a molecule from the bilayer center, on the order of tens of kJ/mol. The free energy of removal is consistently dependent on the length of the molecule, varying from ~25 kJ/mol for hexane to ~70 kJ/mol for nonacosane. In addition to calculating the energy cost of removing these molecules from the center of the bilayer, it has been observed that long hydrophobic molecules such as alkanes tend to adopt two conformations in the center of the bilayer, either in the plane of the bilayer, between the terminal methyl groups of the lipid tails, or parallel to the bilayer normal, interdigitating with the lipid tails. Further constrained simulations may be used to calculate the rate of exchange between these two modes.

827-Pos Board B706
Monte Carlo Simulations of Sterol Superlattice Mosaics in Bilayers Yield Simultaneous Agreement with Concentration and Chemical Potential Data

Carl S. Helrich[1], Rebecca K. Friesen[1], Kathy A. Steiner[1], Erwin Sucipto[2].

[1]Goshen College, Goshen, IN, USA, [2]Bethel College, Mishawaka, IN, USA.

Multiple experiments suggest that at certain mol fractions ($\chi_{sterol} = C_r$) sterol molecules, cholesterol (chol) and ergosterol (erg), form superlattice (SL) structures occupying particular acyl chain sites in a lipid bilayer. We have constructed a theoretical model, which we have tested successfully against our own nystatin-erg channel data and the fluorescence measurements of sterol concentration [1]. Our Monte Carlo (MC) simulations show that mosaics of SLs, with structures strongly dependent on χ_{sterol} , form in bilayers for all χ_{sterol} studied. Here we successfully test the model against the chemical potential data using Kirkwood's coupling parameter method. Holding all interaction energies (sterol/sterol, sterol/lipid) constant the model successfully predicts simultaneously the observed increase in chemical potential [2] and no marked increase in sterol concentration at $\chi_{sterol} = 0.4$ [1].

1. Chong, P.L-G., and M. Olsher. 2004. Fluorescence Studies of the Existence and Functional Importance of Regular Distributions in Liposomal Membranes. Soft Mat. 2:85-108.
2. Ali, M.R., K.H. Cheng, and J. Huang. 2007. Assess the nature of cholesterol-lipid interactions through the chemical potential of cholesterol in phosphatidylcholine bilayers. PNAS 104:5372-5377.

828-Pos Board B707
The Temperature Dependence And Quantized Nature Of The Lipid Membrane Permeability

Andreas Blicher, Katarzyna Wodzinska, Thomas Heimburg.

Niels Bohr Institute, Copenhagen, Denmark.

We investigate the permeability of phospholipid membranes for fluorescence dyes and ions. We find that the permeability is strongly enhanced close to the chain melting transition of the membranes. Close to transitions, fluctuations in area and compressibility are high, leading to an increased likelihood of spontaneous lipid pore formation. Fluorescence Correlation Spectroscopy (FCS) reveals the permeability for rhodamine dyes across 100 nm vesicles. Using FCS, we find that the permeability of vesicle membranes for fluorescence dyes is within error proportional to the excess heat capacity. To estimate defect size we measure the conductance of solvent-free planar lipid bilayer. Microscopically, we show that permeation events appear as quantized current events. Furthermore, we demonstrate that general anaesthetics lead to a change in membrane permeability that can be predicted from their effect on heat capacity profiles. Depending on temperature, the permeability can be enhanced or reduced. We demonstrate that anaesthetics decrease channel conductance and ultimately lead to 'blocking' of the lipid pores in experiments performed at or above the chain melting transition. Our data suggests that the macroscopic increase in permeability close to transitions and microscopic lipid channel formation are the same physical process.

829-Pos Board B708
Headgroup and Interfacial Hydration in Some Headgroup-Modified Analogues of Dimyristoylphosphatidylethanolamine: A DSC and FTIR Spectroscopic Study

Ruthven N. Lewis[1], **Maria Frias**[2], John R. Silvius[3], Ronald N. McElhaney[1].

[1]University of Alberta, Edmonton, AB, Canada, [2]University of Buenos Aires, Buenos Aires, Argentina, [3]McGill University, Montreal, QC, Canada.

The thermotropic phase behavior and organization bilayers composed of dimyristoyl phosphatidylethanolamine and some of its headgroup-modified analogues were investigated by differential scanning calorimetry and by fourier-transform infrared spectroscopy. As expected, chemical modification of the lipid headgroup resulted in changes in the gel/liquid-crystalline phase transition temperatures of the analogues that were positively correlated with the capacity for inter headgroup hydrogen-bonding to the phosphate moiety, and negatively correlated with the size of the headgroup substituent. However, such changes also drastically altered the nature of the lamellar crystalline phases formed by these compounds as well as the lateral packing interactions between the hydrocarbon chains. Moreover, although the chemical modifications of the polar headgroup did not alter the net charge of the lipid headgroup, they did nevertheless, markedly alter the susceptibility of these membranes to penetration by interfacially active molecules such as antimicrobial peptides. Our studies suggest that many of the effects of the chemical modification of the lipid headgroup can be rationalized in terms of their effects on the hydration of, and hydrogen-bonding interactions in the polar/polar interfacial regions of the lipid bilayer.

830-Pos Board B709
Giant vesicles under oxidative stress

Karin A. Riske[1], Tatiane Sudbrack[2], Nathaly L. Archilha[2], Adjaci U. Fernandes[2], André P. Schroder[3], Carlos M. Marques[3], Maurício S. Baptista[2], Rosangela Itri[2].

[1]Universidade Federal de Sao Paulo, Sao Paulo, Brazil, [2]Universidade de Sao Paulo, Sao Paulo, Brazil, [3]Institut Charles Sadron, Strasbourg, France.

We use optical microscopy to observe giant unilamellar vesicles (GUVs) during the initial steps of membrane oxidative damage. A photo-sensitive molecule consisting of a porphyrine linked to a lipid headgroup (PE-porf) is incorporated in the lipid bilayer, thus generating singlet oxygen at the bilayer surface. This system enables studies on the effects of lipid peroxidation in a more controlled fashion, allowing threshold determinations of membrane oxidative damage. We show that irradiation of GUVs containing PE-porf induces immediate increase in vesicle surface area, with accompanying morphological changes. Because porphyrin is rapidly photo-bleached, the oxidation process is halted during the initial formation of hydroperoxides and membrane disruption is avoided. The increase in surface area is quantified as a function of irradiation time and the amount of PE-porf incorporated. The increase in area was related to the migration of hydroperoxides to the polar interface. The coupling of lipid peroxidation to morphological changes of natural membranes may be involved in the activation of different cellular responses to oxidative stress.

831-Pos Board B710
Miscibility Phase Behavior Of GUV Membranes Containing Ternary Mixtures Of PS Lipids, PC Lipids, And Cholesterol

Jake Ashcraft, Sarah Keller.

University of Washington, Seattle, WA, USA.

Vesicles containing ternary mixtures of phospholipids and cholesterol produce coexisting liquid phases over a range of compositions and temperatures.

To date, most studies of membrane phase behavior have used uncharged lipids, which readily produce giant unilamellar vesicles through electroformation. My goal is to study the phase behavior of membranes containing charged lipids such as phosphatidylserine, which is found in the inner leaflet of cell plasma membranes. I present experimental protocols to prepare giant unilamellar vesicles containing ternary mixtures of phosphatidylserine (PS) lipids, phosphatidylcholine (PC) lipids, and cholesterol, based on earlier protocols by Akashi et al [1], Rodriguez et al [2], and Claessens et al [3]. I also detail the phase behavior of membranes of several compositions that incorporate PS lipids.

[1] Akashi et al., ''Formation of Giant Liposomes Promoted by Divalent Cations: Critical Role of Electrostatic Repulsion.'' 74 (1998) 2973-2982.
[2] Rodriguez et al., ''Giant vesicles formed by gentle hydration and electroformation: A comparison by fluorescence microscopy.'' Colloids and Surfaces B: Biointerfaces 42 (2005) 125-130.
[3] Claessens et al., ''Charged Lipid Vesicles: Effects of Salts on Bending Rigidity, Stability, and Size.'' Biophysical Journal 87(6) (2004) 3882-3893.

832-Pos Board B711

Direct Measurement Of Nonideal Mixing In Lipid Membranes

Thomas G. Anderson, Bradley Towey.
Hamline University, Saint Paul, MN, USA.

Lipid components in membranes are known to mix non-ideally, but the thermodynamics of this mixing remains poorly understood. Deviations from ideality may be characterized in part by the heat that is absorbed or released when components mix. This study aims to directly measure the heat of mixing of two phospholipid species in a bilayer membrane, using isothermal titration microcalorimetry. Unilamellar vesicles of 1-palmitoyl-2-oleoyl-sn-glycero-3-phsophocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (POPG) were mixed in a calorimeter cell in the presence of methylated beta-cyclodextrin, which served as a lipid transfer catalyst. We expected on the basis of reduced head-group charge repulsions that mixing of the two lipids would be energetically favorable. The measured heat of mixing for a 1:1 mixture of POPC and POPG is -0.15 kJ/mol.

833-Pos Board B712

Lipid Domains In Giant Vesicles Composed Of Ternary Lipid Mixtures Containing Cholesterol And Their Relationship With Thermodynamic Phases

Laura Rodriguez Arriaga[1], John Ipsen[2], Alejandra Garcia[3], Steffen Härtel[3], Francisco Monroy[1], **Luis A. Bagatolli**[4].
[1]Dept. of Chemistry, Universidad Complutense de Madrid, Madrid, Spain, [2]MEMPHYS- University of Southern Denmark, Odense, Denmark, [3]SCIAN, Faculty of Medicine, University of Chile, Santiago, Chile, [4]Department of Biochemistry and Molecular Biology/MEMPHYS- University of Southern Denmark, Odense, Denmark.

Fluorescence microscopy related techniques provide a powerful tool for direct observation of lipid domains in giant unilamellar vesicles (GUVs) [1]. Using these techniques it was reported that liquid-ordered (lo) - liquid-disordered (ld) phase coexistence can be observed in GUVs composed of cholesterol containing ternary lipid mixtures [1,2]. However, still it is not rigorously established if the lipid domains observed in these GUVs correspond to real thermodynamic phases. Recently we introduced a new method to measure the area fractions of the coexisting lipid domains in GUVs [3]. This novel procedure that involves deconvolution and segmentation of the individual GUV's fluorescence image stacks (including fitting with 3D surface models), allows reconstruction of GUVs 3D structure, permitting to retrieve, at the level of single vesicles, the area fractions of the coexisting lipid domains. The last procedure allowed us to demonstrate quantitatively the accomplishment of the lever rule in GUVs composed of binary phospholipid mixtures displaying solid ordered/liquid disordered domains [3].

In this work we measured the relative areas of the two observed distinct regions (lipid domains) at the reported lo/ld coexisting region in GUVs composed of POPC/DPPC/cholesterol mixtures (approximately 20 different compositions). We explore subsequently if the relationship between the measured areas are consistent with that expected for coexistence of real thermodynamic phases. In particular our method provides a mean of characterizing the tie lines in the lo/ld coexistence region, providing evidences of a connection between what is observed in GUVs and what is predicted from the 3-component phase diagram of the lipid mixture.

1) Bagatolli, L.A, 2006, Biochim Biophys Acta 1758:1541-1556.
2) Veatch, S.L and Keller S.L, 2005, Biochim Biophys Acta. 1746:172-85.
3) M. Fidorra et al., 1198-Pos Biophys. J. 2008 94:1198.

834-Pos Board B713

Bursting Instability of Charged Multicomponent Vesicles Subjected to Electric Pulses

Karin A. Riske[1], Roland L. Knorr[2], **Rumiana Dimova[2]**.
[1]Universidade Federal de São Paulo, São Paulo, Brazil, [2]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

Strong electric pulses applied to neutral phosphatidylcholine (PC) giant vesicles induce the formation of pores, which typically reseal within milliseconds [1]. Here, we study the response of vesicles containing PC and negatively charged phospatidylglycerol (PG) to such pulses. Vesicles composed of 1:1 PG:PC in buffered solution of Hepes and EDTA exhibit the same behavior as observed with PC, namely, the electroporated membrane reseals. Surprisingly, when the medium is changed to a non-buffered solution with or without salt, the vesicles burst and disintegrate to tubular structures after the pulse is applied. Vesicle bursting is abolished when EDTA is present, and recovered with further addition of CaCl2. This suggests that the presence of small amounts (impurities) of multivalent cations (possibly calcium) in the salt and non-buffered solutions is the reason for the membrane instability upon pulse application in the absence of EDTA. In a similar fashion, such impurities were found to induce changes in the thermal behavior of dimyristoyl phosphatidylglycerol [2].

In this work, we use fast digital camera and confocal microscopy to observe the dynamics of vesicle rupture and the membrane reorganization after the applied pulse. The nature of this structural rearrangement is poorly understood. Vesicles made of lipid extract from human plasma membranes behave in the same fashion. Thus, the reported bilayer reorganization may also occur to a certain degree in the membrane of electroporated cells. Studying the electric-pulse response and reorganization of charged model membranes in different medium conditions is a significant and necessary step towards understanding the long pore lifetime in electroporated cells, which allows the transport of drug and DNA molecules.

1. Riske, K.A., and R. Dimova. 2005. Biophys. J. 88:1143-1155.
2. Riske, K.A., H.-G. Döbereiner, and M.T. Lamy-Freund. 2003. J. Phys. Chem. B 107:5391-5392.

835-Pos Board B714

Effects Of Sodium Halide Solutions Of High Concentrations On Bending Elasticity Of POPC GUVs

Héléne Bouvrais[1,2], Philippe Méléard[2], Tanja Pott[2],
John Hjort Ipsen[1].
[1]Memphys Center for Biomembranes, Odense, Denmark, [2]UMR6510-CNRS, Rennes, France.

The Hofmeister series for salt solutions appears in many contexts of biophysics and physical chemistry, e.g. enzymatic activity, stability of biomolecules like the proteins, polymer folding and interfacial tension, while its effect on membrane mechanical properties has only been sparsely explored. With a newly established electroformation technique [1], we have been able to form GUVs (Giant Unilamellar Vesicles) in presence of high salt concentrations. In this study, we have explored the electroformation technique to form POPC GUVs in presence of different sodium halide solutions, and using the flickering technique, we have measured their effects on the bending elastic modulus of POPC bilayers.

[1] Pott T., H. Bouvrais, P. Méléard, ''Giant unilamellar vesicle under physiologically relevant conditions'', Chemistry and physics of lipids, Vol. 154, 2008, pp: 115-119.

836-Pos Board B715

Cholesterol Perturbs Lipid Bilayers Non-Universally

Jianjun Pan, Thalia T. Mills, Stephanie Tristram-Nagle, John F. Nagle.
Carnegie Mellon University, Pittsburgh, PA, USA.

Cholesterol is well known to modulate the physical properties of biomembranes. Using modern x-ray scattering methods, we have studied the effects of cholesterol on the bending modulus K_C, the thickness D_{HH}, and the orientational order parameter S_{xray} of lipid bilayers. We find that the effects are different for at least three classes of phospholipids characterized by different numbers of saturated hydrocarbon chains. Most strikingly, cholesterol strongly increases K_C when both chains of phospholipids are fully saturated but not at all when there are two mono-unsaturated chains.

837-Pos Board B716

The Influence of Sterol Composition on Transbilayer Diffusion Rates

Christine M. Staloch, Benjamin R. Hoffmann, **Benjamin L. Stottrup**.
Augsburg College, Minneapolis, MN, USA.

Cholesterol is a uniquely important sterol to mammalian cell membranes and has been shown to suppress the translocation of lipids between leaflets of a