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

bilayer and therefore plays an important role in maintaining lipid asymmetry. Leaflet composition is regulated by the active transport of lipids by membrane proteins, while thermal diffusion across a membrane tries to randomize the leaflet composition. We have measured the transbilayer diffusion rates for three different sterols over a wide range of compositions. The sterols studied were all cholesterol analogs; including dihydrocholesterol, ergosterol, a component of fungal cell membranes, and stigmasterol, an unsaturated plant sterol. Temperature was varied to determine its influence on transbilayer diffusion rates. We find that sterol structure does have an influence on the rate at which lipids move between bilayer leaflets. Transbilayer diffusion measurements were made using a sodium dithionite assay to monitor the location of lipid analogues within DMPC/sterol liposomes.

838-Pos Board B717

Cholesterol Flip-flop And Chemical Potential In A Systematic Set Of Lipid Bilayers

W.F. Drew Bennett[1], Justin L. MacCallum[2], Marlon J. Hinner[3], Siewert J. Marrink[4], D. Peter Tieleman[1].
[1]University of Calgary, Calgary, AB, Canada, [2]University of California San Francisco, San Francisco, CA, USA, [3]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [4]University of Groningen, Groningen, Netherlands.

Cholesterol is a necessary component of animal cellular membranes. The concentration of cholesterol varies from 0-5 mol% in the endoplasmic reticulum to 25-40mol% in the plasma membrane. Thermal fluctuations cause cholesterol to move normal to the plane of the bilayer. At the extremes, cholesterol can translocate across the bilayer (flip-flop) and diffuse from the bilayer into water (desorption). We have used atomistic and coarse grained molecular dynamics computer simulations to investigate the partitioning of cholesterol through a systematic set of lipid bilayers. Atomistic simulations provide detailed analysis, while inexpensive coarse grained simulations allow more bilayers to be investigated and longer time scales to be sampled. From the coarse grained simulations, cholesterol flip-flop was directly observed, and the rate matched our estimate from the free energy barrier. We find the rate of cholesterol flip-flop is fast and strongly dependent on the structure of the bilayer. The rate of flip-flop is on the microsecond range in fluid, disordered poly-unsaturated bilayers, and on the second range in rigid, ordered bilayers with high cholesterol content. The chemical potential of cholesterol in the bilayer compared to water is equal to our free energies of desorption. We can infer the relative affinity of cholesterol for the bilayers by comparing the chemical potentials. We find cholesterol prefers more ordered and rigid bilayers with saturated acyl tails, and high cholesterol content. Cholesterol has the lowest affinity for poly-unsaturated lipids.

839-Pos Board B718

The Behavior of Two Oxidized Derivatives of Cholesterol in Model Membranes

Evan Mintzer, Grace Charles.
Stern College for Women, New York, NY, USA.

Cholesterol's role in ordering lipid membrane domains is well known. Even subtle changes in the structure of this sterol greatly affect the biophysical dynamics of membranes, usually because of perturbations in the interactions between the sterol and other membrane lipids that chemical modifications cause.

Cholesterol oxidation products (oxysterols), which result from enzymatic and non-enzymatic mechanisms, are cytotoxic and found in atherosclerotic plaques. Previous studies have shown that the membrane properties of oxysterols vary, depending on the specific site of the oxygen-containing moiety. In this study, we examined the interactions of two oxysterols, one formed through non-specific oxidation (7-ketocholesterol), and one produced enzymatically (25-hydroxycholesterol) with two common membrane lipids, 1-palmitoyl-2-oleoyl-*sn*-phosphocholine (POPC) and brain-derived sphingomyelin.

Analysis of force-area isotherms obtained by compression of pure sterol monolayers and of binary monomolecular films at the air-water interface, comprised of varying mole fractions of POPC or sphingomyelin and either oxysterol, reveals significant differences in surface behavior with respect to each other and to native cholesterol. Both oxysterols condensed POPC and sphingomyelin films to a lesser degree than cholesterol, and an expansion of sphingomyelin films was observed with low mole fraction 7-ketocholesterol. Additionally, surface compression moduli data obtained from the force-area isotherms reveal a decreased ability of both oxysterols to mitigate the phase transition of sphingomyelin compared to cholesterol. The changes of membrane behavior in the presence of oxysterols reported here suggest a relation of their toxicity to the propensity of lipids membranes to form liquid-ordered domains (rafts).

840-Pos Board B719

A Calorimetric and Spectroscopic Comparison of the Effects on Ergosterol and Cholesterol on the Thermotropic Phase Behavior and Organization of Dipalmitoylphosphatidylcholine Bilayer Membranes

David A. Mannock, Ruthven N.A.H. Lewis, **Ronald N. McElhaney**.
University of Alberta, Edmonton, AB, Canada.

We performed comparative DSC and FTIR spectroscopic measurements of the effects of cholesterol (Chol) and ergosterol (Erg) on the thermotropic phase behaviour and organization of DPPC bilayers. Erg is the major sterol in the biological membranes of yeasts, fungi and many protozoa. It differs from Chol in having two additional double bonds, one in the steroid nucleus at C7-8 and another in the alkyl chain at C22-23. Erg also has an additional methyl group in the alkyl chain at C24. Our DSC studies indicate that the incorporation of Erg is more effective than Chol is in reducing the enthalpy of the pretransition. At concentrations below 10 mol%, Erg is also more effective than Chol in reducing the enthalpies of both the sharp and broad components of main phase transition. However, at sterol concentrations from 30-50 mol %, Erg is generally less effective at reducing the enthalpy of the broad components and does not completely abolish the cooperative hydrocarbon chain-melting phase transition at 50 mol% as does Chol. Moreover, in this higher ergosterol concentration range there is no evidence of the formation of ergosterol crystallites or of the lateral phase separation of Erg-enriched phospholipid domains. Our FTIR spectroscopic studies demonstrate that Erg incorporation produces a less tightly packed bilayer than does Chol which is characterized by increased hydration in the glycerol backbone region of the DPPC bilayer. These and other results indicate that Erg is less miscible in DPPC bilayers at higher concentrations than is Chol.

841-Pos Board B720

Schiff Base Formation Between The Cholesterol Oxidation Product 3β-hydroxy-5-oxo-5,6-secocholestan-6-al And Amino Phospholipids

Ellen J. Wachtel, Diana Bach, Israel R. Miller.
Weizmann Institute of Science, Rehovot, Israel.

The keto-aldehyde 3β-hydroxy-5-oxo-5,6- secocholestan-6-al is formed by the oxidation of cholesterol with ozone. This oxidized form of cholesterol is associated with a number of pathological conditions including atherosclerotic plaques, Alzheimer's and Parkinson's diseases. We have shown earlier that the compound can react covalently with the amino group of phosphatidylethanolamine to form a Schiff base . Here, using a spectroscopic technique, we determine the kinetics of the Schiff base formation between 3β-hydroxy-5-oxo-5,6- secocholestan-6-al and dimyristoylphosphatidylethanolamine in both the gel and liquid crystalline states of the phospholipid. The activation energies of this reaction in the two states are also calculated. In addition, we determine that a Schiff base can also be formed with the amino group of phosphatidylserine, albeit with slower kinetics. These findings are significant as they show that oxidized cholesterol can react covalently not only with the amino groups of proteins, but also with the amino groups of phospholipids, potentially influencing the structure of biological membranes.

842-Pos Board B721

A Comparison Of Ceramide And Ceramide-1-phosphate Miscibility In Phosphatidylcholine Bilayers

Michael R. Morrow[1], Anne Helle[2], Joshua Perry[1], Ilpo Vattulainen[3], Susanne K. Wiedmer[2], Juha M. Holopainen[2].
[1]Memorial University of Newfoundland, St. John's, NL, Canada, [2]University of Helsinki, Helsinki, Finland, [3]Helsinki University of Technology, Helsinki, Finland.

Sphingolipids are key lipid regulators of cell viability: ceramide is one of the key molecules in inducing programmed cell death (apoptosis), whereas other sphingolipids, such as ceramide 1-phosphate, are mitogenic. The phase behavior of bilayers comprising binary mistures of N-hexadecanoyl-D-erythro-ceramide (C_{16}-ceramide) or N-hexadecanoyl-D-erythro-ceramide-1-phosphate (C_{16}-ceramide-1-phosphate; C_{16}-C1P) with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were studied using differential scanning calorimetry (DSC) and deuterium nuclear magnetic resonance (^{2}H-NMR). Partial phase diagrams (up to a sphingolipid mole fraction of X=0.40) were constructed for both mixtures. For C_{16}-ceramide-containing bilayers DSC heating scans at X_{cer}=0.025 showed a complex structure of the main phase transition peak suggestive of lateral phase separation. The transition width increased significantly upon increasing X_{cer}, and the upper phase boundary temperature of the mixture shifted to ~65°C at X_{cer} = 0.40. The temperature range over which ^{2}H-NMR spectra of C_{16}-ceramide/DPPC-d_{62} mixtures displayed coexistence of gel and liquid crystalline domains increased from ~10° for X_{cer}=0.1 to ~21° for X_{cer}=0.4. DSC and ^{2}H-NMR observations of C16-C1P/DPPC mixtures at corresponding concentrations indicated that two-phase coexistence was limited to significantly narrower ranges of temperature for mixtures containing C16-C1P