

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.

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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-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphorac-(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.

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

Laura Rodriguez Arriaga¹, John Ipsen², Alejandra Garcia³, Steffen Härtel³, Francisco Monroy¹, Luis A. Bagatolli⁴.

¹Dept. of Chemistry, Universidad Complutense de Madrid, Madrid, Spain, ²MEMPHYS- University of Southern Denmark, Odense, Denmark, ³SCIAN, Faculty of Medicine, University of Chile, Santiago, Chile, ⁴Department 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.

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Bursting Instability of Charged Multicomponent Vesicles Subjected to Electric Pulses

Karin A. Riske¹, Roland L. Knorr², Rumiana Dimova².

¹Universidade Federal de São Paulo, São Paulo, Brazil, ²Max 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 phosphatidylglycerol (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

Hélène Bouvrais^{1,2}, Philippe Méléard², Tanja Pott²,

John Hjort Ipsen¹.

¹Memphys Center for Biomembranes, Odense, Denmark, ²UMR6510-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.

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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