

quickly and more precisely, showing that the coupling affects the dynamics. This leaflet coupling is found to heavily influence morphological evolution; in some cases the equilibrium morphological phase observed is very different from what was observed with our simpler monolayer model using similar conditions. We construct a phase diagram of equilibrium morphological phases in the composition space for a few values of the strength of the leaflet coupling. This model has been able to reproduce results found in lipid bilayer experiments probing interleaflet interactions, including the effect of domain registration across leaflets. For the vesicle model, we investigate how an ellipsoidal geometry imposed in the initial conditions affects the phase and morphological evolution.

1821-Pos Board B665**The Ionic State Of Ceramide 1-phosphate Affects Raft Domain Morphology And Fluidity**

Zhiping Jiang, Arne Gericke.

Kent State University, Kent, OH, USA.

Ceramide 1-phosphate (Cer1P) is involved in cell survival, cell proliferation, inflammation and phagocytosis processes. Physiological processes that have been associated with Cer1P have been shown to be in some cases lipid raft dependent. Lipid rafts are proposed to exist in a liquid-ordered state it has been suggested that raft domains are involved in a variety of important biological processes. It has been shown that ceramide forms gel phase domains within the liquid-ordered raft domains and the question arises what kind of phase state Cer1P adopts when immersed in a raft domain. The physicochemical behavior of Cer1P is mainly routed in the protonation state of the phosphate headgroup. To investigate the phase behavior of Cer1P in raft domains, giant unilamellar vesicles (GUVs) composed of POPC/Sphingomyelin/Chol (1:1:1) with different concentrations of Cer1P were studied by fluorescence microscopy at buffers with different pH (pH5, pH7 and pH9). For a pH 7 buffer, the presence of Cer1P disrupted raft domains and induced lipid phase reorganization and the appearance of a Cer1P-enriched gel phase. In contrast to the large platforms reported for ceramide, the presence of Cer1P disrupts rafts. For pH 5 buffer, with increasing concentrations of Cer1P, the domain patterns were totally different from those observed for pH 7 buffer. The Cer1P gel phase disappeared completely and the raft type liquid disordered phase became dominant. In pH 9 buffer, the ability of Cer1P to disrupt rafts was attenuated. These experiments demonstrate that the protonation state of the phosphate headgroup affects the phase behavior of Cer1P within the raft. The headgroup of Cer1P might function as an electrostatic switch that drives the lipid in and out of gel phase domains which may modulate its availability to the relevant proteins.

1822-Pos Board B666**Interdigitation, Domains and Morphology, in Membranes of the Chain Asymmetric C24:1 Ceramide**Sandra N. Pinto¹, Liana C. Silva¹, Rodrigo F.M. de Almeida²,Manuel Prieto¹.¹Centro de Química-Física Molecular and IN, Lisbon, Portugal.²Centro de Química e Bioquímica, Faculdade de Ciências, UL, Lisbon, Portugal.

Ceramide (Cer) is involved in the regulation of several biological processes, such as apoptosis and cell signaling. The alterations induced by Cer in the biophysical properties of membranes are thought to be one of the major routes of Cer action. To gain further knowledge about the alterations induced by Cer, membrane reorganization by the very long chain asymmetric nervonoylceramide (NCer) was studied. The application of an established fluorescence multiprobe approach, together with x-ray diffraction, differential scanning calorimetry, and confocal fluorescence microscopy, allowed the characterization of NCer and the determination of the phase diagram of palmitoyl-oleoylphosphatidylcholine (POPC)/NCer binary mixtures. Nervonoylceramide undergoes a transition from a mixed interdigitated gel phase to a partially interdigitated gel phase at 20°C, and a broad main transition to the fluid phase at 52°C. The solubility of NCer in the fluid POPC is low, driving gel-fluid phase separation, and the binary-phase diagram is characterized by multiple and large coexistence regions between the interdigitated gel phases and the fluid phase. At 37°C, the relevant phases are the fluid and the partially interdigitated gel. Moreover, the formation of NCer interdigitated gel phases leads to strong morphological alterations in the lipid vesicles, driving the formation of cochleate-type tubular structures.

Biophys. J. 2008 95: 2867-2879

1823-Pos Board B667**Interaction of Antimicrobial Oligomers with Lipids Studied by Solid-State NMR**

Weiguo Hu, Abhigyan Som, Gregory N. Tew.

University of Massachusetts, Amherst, MA, USA.

A family of synthetic mimic of antimicrobial peptides (SMAMP), amphiphilic *meta*-phenylene ethynylene (mPE) molecules show a wide range of antimicrobial activity and specificity. The interaction of a specifically active mPE molecule (AMO-2) with mixed DOPE/DOPG lipid was studied by solid-state NMR. The AMO-2 molecules do not preferentially interact more strongly with either lipid component, but rather are well dispersed in the lipid matrix. AMO-2 intimately interacts with all parts of lipid molecules, including head groups. Magic-angle spinning sideband analysis indicated that in samples with co-existing lamellar and inverted hexagonal phases (H_{II}), neither lipid component aggregate in either phase. The presence of AMO-2 molecules causes dynamic disorder in lipid head groups, as evidenced by the broadening of both static and MAS ³¹P spectra. AMO-2 molecules do not massively transform the lamellar lipid into H_{II} phase.

1824-Pos Board B668**Phase Separation in Binary Mixtures of Bipolar and Monopolar Lipid Dispersions Revealed by Solid-State 2H NMR Spectroscopy and Small Angle X-ray Scattering**Andrey Struts¹, David P. Holland², Gabriel Longo², Matthew J. Justice³, Igal Szleifer², Avigdor Leftin¹, Horia I. Petrache³, Michael F. Brown¹, David H. Thompson².¹University of Arizona, Tucson, AZ, USA, ²Purdue University, West Lafayette, IN, USA, ³Indiana University-Purdue University, Indianapolis, IN, USA.

Binary mixtures of C20BAS and POPC membranes have been studied by 2H NMR spectroscopy and small angle X-ray scattering (SAXS) over a wide range of concentrations and at different temperatures. Experiments tested the possibility of formation of phase-separated lipid domains predicted by the mean field theory [1]. Membranes composed of three specifically deuterated C20BAS derivatives [1,1,20,20-2H4]C20BAS, [2,2,19,19-2H4] C20BAS, and [10,11-2H2] C20BAS with protiated POPC and with membranes containing POPC-d31 and fully protiated bolalipid were used in NMR experiments to obtain structural information for the mixture. The 2H NMR spectra of 10,11-2H2-C20BAS:POPC membrane dispersion reveal that the bolalipid is predominantly in the transmembrane conformation at high bolalipid concentrations. At 50 mole percent C20BAS and lower, components appear in the spectra with smaller quadrupolar couplings, most likely indicating the presence of U-shaped conformers. The proportion of U-shaped bolalipids becomes more prominent as the amount of POPC in the membrane increases. However, the transmembrane component is still the dominant bolalipid conformation in the membrane even at 45 °C and 10 mole percent C20BAS, where it accounts for roughly 50% of the bolalipid population. The large fraction of C20BAS transmembrane conformers regardless of the C20BAS:POPC ratio together with POPC-bolalipid hydrophobic mismatch can be explained by co-existence of bolalipid-rich domains separate from the POPC-rich domains. In SAXS experiments only a single distinct lamellar repeat distance was observed, corresponding roughly to the average of bolalipid-rich and POPC-rich domains. These observations are consistent with the presence of microphase-separated domains in the mixed membrane samples. [1] G.S. Longo et al. (2007) Biophys. J. 93, 2609.

1825-Pos Board B669**Interactions of Ceramide and Sphingomyelin Quantified in Mixtures with an Unsaturated Phosphatidylcholine**Bruno M. Castro¹, Rodrigo F.M. de Almeida², Liana C. Silva¹,Manuel Prieto¹.¹CQFM and IN, IST, Lisboa, Portugal, ²CQB, FCUL, Lisboa, Portugal.

Over the past two decades, the discovery of sphingolipid signaling pathways has stimulated research on the biochemistry and biophysics of these molecules. The involvement of membrane biophysical changes is important but difficult to assess. A detailed characterization of these changes can be obtained in systems where the global lipid composition is controlled and known, as in the case of model membranes.

To better understand how sphingolipids modulate the biophysical properties of the membrane, the interactions between palmitoyl-ceramide (PCer) and palmitoyl-sphingomyelin (PSM) were studied in the presence of the fluid and naturally abundant phospholipid palmitoyl-oleoyl-phosphatidylcholine (POPC) in membrane model systems [1]. The use of two fluorescent membrane probes, distinctly sensitive to lipid phases allowed a thorough biophysical characterization of the system. In these mixtures, PCer recruits POPC and PSM in the fluid phase to form extremely ordered and compact gel domains. Gel domain formation by low PCer mol fraction (up to 12 mol %) is enhanced by physiological PSM levels (20-30 mol % total lipid). For higher PSM content, a three-phase situation, consisting of fluid (POPC-rich)/gel (PSM-rich)/gel (PCer-rich) coexistence, is clearly shown. To determine the fraction of each phase a quantitative method was developed. This allowed establishing the complete ternary phase

diagram, which helps to predict PCer-rich gel domain formation (e.g., upon sphingomyelinase action) and explains its enhancement through PSM/PCer interactions.

[1] Castro, B. M., de Almeida, R. F. M., Silva, L. C., Fedorov, A., Prieto, M. *Biophys. J.* **2007**, 93, 1639-1650.

FCT (Portugal) is acknowledged for project funding and research grants.

1826-Pos Board B670

Probing The Microstructure Of Biomaterials With Positrons

Petri Sane¹, Filip Tuomisto¹, Ilpo Vattulainen¹, Empu Salonen¹, Juha Holopainen².

¹Helsinki University of Technology, Espoo, Finland, ²University of Helsinki, Helsinki, Finland.

In this work we present a novel and promising tool for characterizing the microstructural changes in biomaterials e.g. lipid bilayers. Positron annihilation lifetime spectroscopy (PALS) is a widely used tool to study atomic scale defects in semiconductors [1] and routinely used to study the voids in polymer materials [2]. Applying PALS to study biomaterials is uncommon and until recent years mostly unheard of, though preliminary studies were performed in early 1980's [3]. Through the increased understanding of the biomolecular materials, results from PALS experiments can now be compared with simulations and further analysis of the results is possible. In biomolecular material, a thermalized positron forms a meta-stable bound state, Positronium, with an electron from the material. An ortho-Positronium-atom can be applied as a probe, because the Positronium lifetime in the material is strongly affected by the free volume characteristics of the probed material.

Our study combines the experimental results achieved with PALS and atomistic MD simulations. Preliminary PALS measurements were performed with DPPC and POPC-lipid bilayers, complemented with MD simulations [4]. The results from both methods are in full agreement with each other and thus showing PALS as a viable tool to study the free volume changes, or the changes in hydrocarbon tail dynamics inside the lipid bilayer.

As well as studying manufactured lipids with PALS, also *in vivo* studies of organic biomaterial are possible, such as studying the changes in internal free volume/dynamics of a mammalian lens and lipid membranes separated from lenses.

[1] F. Tuomisto et al, *Phys. Rev. Lett.* 93 (2004).

[2] O. E. Mogensen, *Positron annihilation in Chemistry* (Springer-Verlag, Heidelberg, 1995).

[3] Y.Y. Wang et al, *J. Am. Chem. Soc.* 105 (1983).

[4] P. Sane et al "Probing Biomembranes with Positrons", submitted to *J. Am. Chem. Soc.* 15.09.2008.

1827-Pos Board B671

Membrane Flow Patterns In Multicomponent Giant Vesicles Induced By Alternating Electric Fields

Margarita Staykova, Reinhard Lipowsky, Rumiana Dimova.

Max Planck Institute of Colloids and Interfaces, Golm, Germany.

Electric fields are widely used to manipulate cells, viruses, vesicles and cell organelles, e.g. by electroporation, electrofusion, electrophoresis, dielectrophoretic displacement, trapping, sorting, etc. Although the effects of electric fields on lipid membranes have been extensively studied, some basic phenomena have still remained unnoticed. Here, we show for the first time that alternating electric (AC) fields may induce pronounced membrane flows in giant lipid vesicles. This phenomenon occurs in most chambers and conditions used for electric manipulation, where the vesicles experience inhomogeneous fields, due to screening by neighbors, sedimentation, chamber geometry, etc. We use multicomponent lipid vesicles with fluorescently labeled intramembrane domains to visualize the flow. This approach for visualization of membrane dynamics may turn out to be very helpful for studies on membrane behavior in vesicles subjected to shear flows or mechanical stresses. The influence of field parameters and media properties on the lipid flow will be discussed and a mechanism based on finite element calculations will be proposed. The reported phenomenon lead to important questions about the effects of electric fields on membranes and about the hydrodynamic coupling of the membrane to the internal and external fluid media. Finally, the AC-field induced membrane flow has many potential applications in microfluidic technologies as well as for lipid mixing, trapping and displacement, as will be demonstrated.

1828-Pos Board B672

Effective Lifetime Of Membrane Tethers Formed By Multiple Contacts Obeys A Generalized Bell Model

Marius C. Staiculescu¹, Mingzhai Sun², Imre Derenyi³, Gabor Forgacs¹.

¹University of Missouri Columbia, Columbia, MO, USA, ²Princeton University, Princeton, NJ, USA, ³Eotvos Roland University, Budapest, Hungary.

Circulating cells, upon exiting the blood flow, are likely to be slowed down by nanotubular membrane tethers. These structures form through multiple contacts between the circulating cell and the endothelium. The efficacy of tether-mediated slowing down depends on the lifetime of the complex bond connecting the circulating cell to the endothelium, which is usually related to elementary receptor-ligand bonds (i.e. selectin-PSGL1). However, as the number of these elementary bonds is not known this relationship is highly non-trivial. Here we introduce the notion of the effective tether bond and study its lifetime *in vitro*. Specifically, we extract multiple tethers from microvilli presenting cells with constant force, generated by magnetic tweezers and transduced to the cell through cell-sized magnetic beads with different surface properties. We demonstrate that the stochastic effective lifetimes of these tethers are exponentially distributed and the parameters characterizing this distribution obey an appropriately generalized Bell model. We determine the maximum likelihood estimates of these parameters, such as force-free dissociation constant and reactive compliance. We find that their values differ significantly from corresponding typical single-molecular values, reflecting the fact that effective tether bonds are complex. We check the consistency of our methods using computer generated synthetic data. We employ this method to gain insight into the progression of atherosclerosis by studying pathological changes in the endothelial cell membrane. Specifically, we investigate how the cholesterol content of the membrane impacts the lifetime of adhesive (e.g. selectin-ligand) bonds formed by tethers.

This work was supported by NIH-HL64388 (G.F.), the American Heart Association (M.S.) and the Hungarian Science Foundation -K60665 (I.D.)

1829-Pos Board B673

Structural and Dynamic Markers of Membrane Osmotic Stress From X-Ray Scattering and Solid-State 2H NMR

Avigdor Leftin¹, Matthew J. Justice², Jacob G. Kinnun¹, Horia I. Petrache², Michael F. Brown¹.

¹University of Arizona, Tucson, AZ, USA, ²Indiana University-Purdue University, Indianapolis, IN, USA.

Osmotic membrane deformation is one of the most important determinants of biomembrane structure and dynamics since it leads to an alteration of the physiological membrane function [1]. Collective motions within lipid membranes are governed by molecular-scale interactions that are manifested in bilayer material properties. One way to investigate this emergence of material properties over mesoscopic distances is to measure the biomembrane dynamics in the extreme limit of low hydration [2]. To quantify both the structure and dynamic properties of the lipids in this high osmotic stress regime it is useful to apply both 2H NMR spectroscopy and small-angle X-ray scattering. 2H NMR is sensitive to dynamic fluctuations accessed in nuclear spin relaxation experiments, while X-ray scattering provides precise measures of the membrane structural properties [3]. We find that hydration to only a few water molecules per lipid, either gravimetrically or through use of osmolytes, results in large differences in the properties of membranes as observed in the NMR and X-ray experiments. Changes of the 2H NMR acyl chain order parameters SCD and relaxation rates RIZ of multilamellar phospholipid dispersions in the liquid-crystalline state at extremely low hydration levels are mirrored by reduction in inter-bilayer D-spacings as detected by small-angle X-ray scattering. Our results demonstrate that in the regime of high osmotic stress membrane dynamics become increasingly sensitive to small changes in the number of waters per lipid. These changes correspond to an alteration of dynamic fluctuations indicative of collective lipid interactions that are mediated by the water content of the system.

[1] R.P. Rand, V.A. Parsegian, (1989) *BBA* 988, 351-376.

[2] M.F. Brown et al, (2002), *JACS* 124, 8471-8484.

[3] H.I. Petrache, M.F. Brown, (2007), *Methods in Membrane Lipids*, Humana Press, pp. 339-351.

1830-Pos Board B674

Hydrodynamic Extrusion Of Membrane Nanotubes From Neuroendocrine Bon Cells: Role Of Membrane Trafficking Sébastien Kremer.

Institut Curie, Paris, France.

Dynamics of extrusion of membrane nanotubes from giant vesicles (GUVs) and red blood cells (RBCs) are well understood. In GUVs, extrusion dynamics (tether length as a function of time) are governed by the membrane tension, whereas for RBCs adhesion of the plasma membrane (PM) to the cytoskeleton dominates. In the case of cells with endomembranes extrusion dynamics are complicated by lipid trafficking to and from the PM. Constitutive fusion of vesicles with the PM (exocytosis) continuously adds membrane to the PM while the reverse process of membrane retrieval (endocytosis) helps maintaining a stable steady-state cell surface area. In addition, some cells are capable of calcium-regulated secretion in which specialized secretory vesicles await a trigger