

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