

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.

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1826-Pos Board B670

Probing The Microstructure Of Biomaterials With Positrons

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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. Mogensén, *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

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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

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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.

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1829-Pos Board B673

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

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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

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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

signal for undergoing rapid fusion with the PM within seconds. The excess membrane added to the PM is retrieved via compensatory endocytosis on a longer time-scale (minutes). We have been using endocrine BON cells, a cell line derived from a human carcinoma tumor, which are capable of calcium-regulated secretion. Secretion is stimulated, almost instantly during tether extrusion, by UV-uncaging of a photolabile calcium chelator loaded into the cytosol. Thus, a significant transient membrane addition to the PM can be turned on at will and the consequences for extrusion dynamics can be studied.

We observe mainly two types of response upon stimulation: a sudden jump in tether length L of $\sim 10 \mu\text{m}$ followed by an increased extrusion velocity, or a sudden increase of slope. Both responses are consistent with exocytotic addition of area to the PM. However, only a fraction of such responses are well-correlated with UV-uncaging of intracellular calcium. This suggests exocytosis, or other mechanisms of membrane addition to the PM may independently be triggered by other means, possibly by tether pulling itself.

Membrane Fusion

1831-Pos Board B675

Molecular Mechanisms of Vesicle Fusion

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We have performed large scale molecular dynamics simulations using coarse-grained lipid models to study the fusion of vesicles. Our earlier work showed that lipid tail splay played an important role in the first lipids to cross from one vesicle to another. In this work we study the effect of different lipid types on the molecular mechanism of the initial fusion events. Lipid types studied include phosphatidylcholine and phosphatidylethanolamine lipids. In order to examine the importance of lipid splay, lipids with symmetric and asymmetric tail lengths have been studied. We calculate the free energy barrier to fusion by using the umbrella sampling method to determine the potential of mean force as a function of the distance between the center of mass of the two vesicles. We will discuss the comparison of the different curves for the different lipid types and the role of molecular mechanism(s) in vesicle fusion. We will also discuss how the interactions between lipid molecules influence fusion.

1832-Pos Board B676

Imaging Single Virus Fusion Reveals the HIV-1 Entry Pathway

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Viruses whose fusion proteins are activated by interactions with cellular receptors at neutral pH, including HIV-1, are assumed to fuse directly with the plasma membrane. However, direct virus fusion at the cell surface has not been explicitly demonstrated. To differentiate between HIV-1 fusion with the plasma membrane (PM) and with endosomal membrane (EM), we performed time-resolved single virus imaging. Pseudoviruses bearing HIV-1 Env glycoprotein were generated and co-labeled with a content marker (Gag-GFP) and a red lipophilic membrane dye. Upon virus maturation, the Gag-GFP is cleaved by viral protease yielding a smaller GFP-tagged fragment that is readily released from virions permeabilized with saponin. This marker thus provided a convenient means to detect small pore formation during virus-cell fusion. Double-labeled viruses were adhered in the cold to HeLa-derived cells expressing CD4 and coreceptors. Fusion was triggered by shifting to 37°C and monitored by laser scanning confocal microscopy. Imaging of single HIV-cell fusion revealed the occurrence of both PM and EM fusion events. Fluorescent viruses undergoing PM fusion transferred their lipid marker (hemifusion), but not content marker (full fusion). By contrast, full fusion with an endosome was consistently observed. These EM fusion events were manifested in disappearance of a content marker, but not the lipid dye due to its limited dilution in an endosomal membrane. These results demonstrate that, contrary to a common perception, HIV-1 enters HeLa-derived target cells by receptor/coreceptor-mediated endocytosis followed by fusion with endosomes and delivery of viral nucleocapsid into the cytosol.

1833-Pos Board B677

The Gaussian Curvature Elastic Energy of Membrane Fusion Intermediates, and a Possible Mode of Action of Fusion-Mediating Proteins

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Calculating the Gaussian curvature elastic energy of fusion intermediates requires knowledge of the Gaussian curvature elastic modulus, κ . κ can be measured for lipids that form Q_{II} phases. It is shown that one can estimate κ for non- Q_{II} phase lipids by studying phase behavior of lipid mixtures. κ is between -5 and -10 $k_B T$ for biological lipid compositions. The Gaussian curvature elastic energy of fusion intermediates is positive and \geq the total bending energy cal-

culated previously: it increases the total energy of fusion intermediates by 100 $k_B T$ or more. This large contribution makes the predicted intermediate energies compatible with observed lipid phase behavior in excess water. An order-of-magnitude fusion rate equation is used to show that a current theory now predicts stalk energies that are slightly too large, by about 30 $k_B T$, to rationalize the observed rates of stalk-mediated processes in pure lipids. Despite this discrepancy, when the effect of κ is included, current models of fusion intermediate energy can make semi-quantitative predictions about how proteins mediate biomembrane fusion. Fusion-mediating proteins must lower the stalk energy by several tens of $k_B T$ relative to lipidic stalks. One way proteins could do this is by altering the elastic constants of the patches of monolayer that fuse, by inserting peptides. Preliminary data using fusion peptides and membrane-spanning peptides is compatible with this role for peptides. The energies of stalks, fusion pores, rhombohedral (R) phase and Q_{II} phase relative to L_α phase all depend on approximately the same assembly of monolayer elastic constants; ($2k_m \delta J_s - \kappa$). Thus the influence of peptides on stalk and fusion pore energy can be studied by measuring the effects of peptides on R and Q_{II} phase stability (Siegel, Biophys. J.; Dec. 2008).

1834-Pos Board B678

Determination of Free Energy Barriers to Initial Fusion of Vesicles

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The fusion of two vesicles is studied by means of molecular dynamics simulations using coarse-grained lipid models. We use the umbrella sampling method to determine the potential of mean force as a function of the distance between the center of mass of two vesicles. The free energy barrier to the initial fusion step between two vesicles is determined from the peak in the PMF curve calculated at a center of mass distance just prior to the initial fusion step. The two CG lipid models used were the original model by Marrink, de Vries, and Mark (J. Phys. Chem. B 2004, 108,750) and its recently improved and extended version, the MARTINI model. We find that the free energy barrier for the initial fusion event varies by more than an order of magnitude between the two models. The source of the difference is found in the greater repulsive character of the interaction between the hydrophobic tail particles and the charged head-group particles in the MARTINI model compared to the older model. This interaction results in the lipid tails being confined to the hydrophobic region of the vesicle to a greater extent and the splay of the lipid tails being limited. These factors reduce the probability of triggering an initial fusion event.

1835-Pos Board B679

Insights Into The Energetics Of Neuronal SNARE Complex Formation

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Eukaryotic cells transport material between intracellular compartments by vesicles that bud from a donor and afterwards fuse with a target organelle. Best-studied is the molecular machinery that drives the Ca^{2+} -dependent release of neurotransmitters from synaptic vesicles. Key players in the exocytotic fusion are three proteins synaptobrevin 2, syntaxin 1a, and SNAP-25. Synaptobrevin resides in synaptic vesicles, whereas syntaxin and SNAP-25 are anchored in the plasma membrane. They belong to the so-called SNARE protein family, which members are involved in all other vesicle fusion steps. SNARE proteins are tail-anchored membrane proteins that assemble into a stable membrane-bridging complex. As SNARE assembly is accompanied by extensive structural rearrangements from mostly unstructured monomers into a tightly packed parallel four-helix bundle, it is thought that zipper-like formation of the SNARE bundle between opposing membranes provides the energy that drives fusion. It is unclear, however, whether the assembly energy indeed suffices for membrane merger. Unfortunately, a marked hysteresis in the folding and unfolding transition of the SNARE complex prevents the direct determination of the free energy of assembly. We have now investigated the assembly process by isothermal titration calorimetry. We found that the structural changes upon assembly are reflected in extremely large favorable enthalpy changes, counterbalanced by a large positive entropy change. Moreover, as SNARE complex is essentially irreversible, we made use of the fact that assembly in vitro proceeds in discrete steps. This allowed us to assess the energetics of each assembly step individually.

1836-Pos Board B680

Clustering of Syntaxin-1A in Model Membranes is Modulated by Phosphatidylinositol-4,5-bisphosphate and Cholesterol

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Syntaxin-1A is part of the SNARE complex that forms in membrane fusion during neuronal exocytosis of synaptic vesicles. Together with SNAP-25, the single-span transmembrane protein syntaxin-1A forms the receptor complex on