

776-Pos Board B655**Plasma Membrane Area Increases With Spread Area By Exocytosis Of GPI Anchored Protein Compartment**

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The mechanism by which cells control plasma membrane (PM) area is poorly understood. Changes in PM area cannot arise from stretching the membrane. This model would predict that membrane tension increases and limit the shape change induced by cell spreading. However, we found that PM tension decreased during spreading, indicating that PM area increased. Accordingly, exocytosis increased PM area by 40-60% during spreading. Moreover the increase in PM area was proportional to the spread area. Golgi, lysosomes and glycosylphosphatidylinositol-anchored protein vesicles (GPI vesicles) exocytosed during spreading, but no fusion of endoplasmic reticulum or transferrin receptor containing vesicles was detected. Microtubule depolymerization blocked lysosome and Golgi exocytosis, but not GPI vesicle exocytosis and PM area increase. We propose that the dramatic increase in PM area during spreading originates selectively from a recycling pool of GPI-anchored protein vesicles.

777-Pos Board B656**Diffusion-Matched and Spectrally-Discrete Lipophilic Probes for Neuronal Tracing***Maria Hansen¹, Jeff Tonniges¹, Jeremy Duncan², Matthew Bassett¹, Bernd Fritschsch², Brian Gray³, Michael Nichols¹.¹Creighton University, Omaha, NE, USA, ²University of Iowa, Iowa City, IA, USA, ³Molecular Targeting Technologies, Inc., West Chester, PA, USA.

Lipophilic fluorescent dyes enable the tracing of neural networks by diffusing laterally between nerve cell membranes. Because they do not require gene expression for labeling, these dyes can be advantageous for studies of both wild-type and mutant mice. To establish the neural connections that are made during development, a diffusion-matched set of spectrally distinct dyes is needed. A set with green, red and far-red fluorescence emission have previously been described [1]. Now, additional near-infrared and violet candidates have been developed. To optimize sequential six-color imaging protocols, we have measured the absolute multiphoton cross section spectra for these dyes. By combining two-photon and confocal microscopy, the entire set can be imaged using a single Ti:S laser. In the environment of a peripheral nerve fiber, the diffusion characteristics of dyes with varied hydrocarbon chain lengths and fluorescent head groups are determined by FRAP and distance measurements. By fitting the data to an anomalous-diffusion model, the time-scaling exponents and transport coefficients can be compared. Finally, we consider how the mechanism of lipophilic dye transport in fixed and living cells can be elucidated by transcellular diffusion from individually labeled cells within an interconnected network.

[1] H. Jensen-Smith et al., IMMUNOL. INVEST, 36(5-6): 763-789, 2007.

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778-Pos Board B657**Liposome Steady-state Anisotropy Of E.coli Total Lipid Extracts: A Tool To Better Understand Antibiotic Translocation In A Bacterial Environment**

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The outer membrane of Gram-negative bacteria is a protective diffusion barrier preventing the free entry of solutes into the periplasm. At the same time, the embedded proteins (Omp's for Outer Membrane Proteins) fulfil a number of tasks that are crucial to the bacterial cell, such as solute and protein translocation, as well as signal transduction. Since a large number of years, a lot of simple membranar models with synthetic lipids such as POPC, POPG, DMPG...allowed scientists to better understand porin insertions and antibiotic translocation/interaction. As any molecular phenomena, it is better to study it in their own environment. With the availability of well characterized E.coli total lipid extract phospholipids, in vitro model membranes such as liposomes and proteoliposomes can be created and antibiotic translocation investigated in a bacterial environment, as close as possible to the existing membranes.

In this study, the effects of porins on the structural order of lipid membranes was investigated by measuring, as a function of temperature, the steady-state fluorescence anisotropy (rS) of TMA-DPH and DPH incorporated into liposomes and proteoliposomes of E.coli total lipid extract phospholipids. DPH, embedded in the bilayer, can give informations about lipid diffusion.

TMA-DPH, anchored at the aqueous interface of the phospholipid bilayer, allow us to better understand Omp's/antibiotic interactions.

By using those probes, we were able to obtain information on the effects caused by the insertion of Omp's into the core and at the interface regions of the bilayer. This information, correlated with other membrane models, can be of considerable interest in establishing a possible relationship between the lipid composition of the phospholipid bilayer surface and antibiotic translocation.

779-Pos Board B658**Laurdan GP Fluctuations In Membranes Of Intact Erythrocytes**

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Lipids in the cell membranes are believed to be organized in micro-domains, known as rafts. If they in fact exist, rafts may provide important boundaries for the organization and sequestering of membrane proteins. The prevailing theory holds that membrane rafts are as small structures (10-200 nm), heterogeneous in size, highly dynamic and sterol- and sphingolipid-enriched domains that compartmentalize cellular processes.

The existence of these small domains is still under debate despite a great deal of work in the area. The most commonly used method to detect rafts is their resistance to solubilization by the nonionic detergent Triton X-100 and sensitivity to cholesterol depletion. These measurements are indirect and potentially open to alternative interpretations. Directly visualizing rafts in living cells has been a difficult task because they are extremely small. Their existence still remains controversial. Here we report the use of a new methodology were two powerful microscopic techniques are applied simultaneously. The first technique, Laurdan Generalized polarization (GP), can differentiate liquid ordered phases from liquid disordered phases based on the water content rather than on the partition properties of the probe. The second technique, Scanning Fluorescence Correlation Spectroscopy quantifies the GP heterogeneities in the membranes. Using these techniques we observed GP fluctuations in the plasma membrane of rabbit red blood cells that can be explained by a model that includes the existence of lipid microdomains, which are heterogeneous in size and mobility. The properties of these GP microdomains are similar to the proposed properties of rafts. Financial support is provided by NIH RR03155.

780-Pos Board B659**Mechanical Pull On A Guest Molecule By A Photoresponsive Lipid Bilayer**Jens A. Lundbaek^{1,2}, Peter Tidemand-Lichtenberg¹, Claus H. Nielsen¹, Johanna M. Kuiper³, Jan B. Engberts³, Bert Poolman³, Salim Abdali¹.¹Department of Physics, Technical University of Denmark, Lyngby,Denmark, ²Weill Medical College of Cornell University, New York, NY,USA, ³Department of Biochemistry, University of Groningen, Groningen, Netherlands.

One of the central challenges in nanotechnology is to develop tools for reversible, mechanical manipulation of non-covalently bound single molecules. Using the gramicidin (gA) channel, we demonstrate that light-induced changes in the mechanical properties of a photoresponsive lipid bilayer allow for optical control of the bilayer disjoining force on a non-covalently bound guest molecule. The gA channel is formed by trans-bilayer association of a monomer from each monolayer in a lipid bilayer. Channel formation in a bilayer where the thickness of the bilayer hydrophobic core exceeds the channel length, involves a local bilayer thinning to match the channel. The bilayer, in response to the deformation, exerts a disjoining force on the channel - the magnitude of which is determined by the bilayer mechanical properties. We studied gA channels in 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC): di-(5-{[4-(4-butylphenyl)azo]phenoxy}pentyl (4-Azo-5P)/n-decane bilayers using single channel voltage clamp techniques. Upon exposure to UV light, the azobenzene moiety in the acyl chains of 4-Azo-5P undergoes a reversible trans-to-cis isomerization, known alter the physical properties of this lipid. By exposing gA channels in DOPC:4-Azo-5P bilayers to alternating visible or UV light, the bilayer disjoining force on the channel was altered such as to cause rapid, reversible changes in channel dissociation rate.

781-Pos Board B660**Study Of The Drug/lipid Interactions Between Cephalosporin Antibiotics And Liposomes By Complementary Techniques**

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The cephalosporins are bactericidal with both gram-positive and gram-negative activity widely prescribed because of their broad spectrum. Even though most of these antibiotics appear to penetrate the outer membrane through porin proteins, we investigated, as a primary step, the interaction at the water-lipid interface. The information of the intrinsic property of these drugs in permeate or simply interact with phospholipid bilayers becomes of great importance for a better understanding of the functions of a lipid component in the membrane