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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. One possibility is that folds in the PM flatten out to follow shape changes. 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.

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Diffusion-Matched and Spectrally-Discrete Lipophilic Probes for Neuronal Tracing*

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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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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.

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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.

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Mechanical Pull On A Guest Molecule By A Photoresponsive Lipid Bilayer

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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.

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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

translocation. For that, the structural order of lipid membranes was investigated by measuring fluorescence polarization of membrane-bound fluorophores such as 1,6-phenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluene sulfonate (TMA-DPH) in the presence and absence of different cephalosporin generations as a function of temperature. Location studies have been also carried out using electron paramagnetic resonance (EPR) spectroscopy, valuable tool for collecting information on the dynamics of lipids and membrane structure. The results obtained suggest that the incorporation of these antibiotics into DMPC and DMPG bilayer does not significantly modify their transition temperature whereas perceptible changes in the cooperativity of the phospholipid transition are observed.

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Inversion of Lipid Bilayer Surface Charge by Trivalent Cations: Probing with Single-channel Conductance and Kinetics

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The conductance of gramicidin A is sensitive to the charge of the lipid bilayer in which it resides. We used this property to probe the effects of lanthanum^{3+}, hexaamminecobalt^{3+}, and spermidine^{3+} on the surface charge of phosphatidylserine (PS) bilayers. Addition of trivalent cations to negatively-charged PS bilayers reduced gramicidin conductance below the conductance seen for neutral phosphatidylcholine bilayers, to a level nearly as low as for positively charged trimethylammonium propane bilayers. This suggests that trivalent cations can overcompensate the negative surface charge of the PS bilayer. Complementary zeta-potential measurements of PS liposomes with trivalent cations also suggested charge inversion. There were differences in the concentrations required to invert charge among the different cations, with lanthanum^{3+} the most potent and spermidine^{3+} the least potent. We also find that the rate of channel formation is sensitive to the surface concentration of permeating ions. Our interpretation is that gramicidin monomers in a bilayer exist in different configurations and that the equilibrium between these configurations depends on the cation binding within monomers. The difference in occupancy of monomers by cations makes channel formation dependent on the surface potential.

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Breakdown of Charged Lipid Asymmetry as a Result of Lipidic Pore Formation

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Negatively charged lipids are usually located in the inner leaflet of the plasma membrane. Their appearance in the outer leaflet is known to correlate with several physiological and pathological conditions in cells. Understanding how membrane lipids lose their asymmetric transmembrane distribution and achieve their nonrandom distribution in cells is a key challenge in cell biology. Negatively charged lipids do not spontaneously exchange between the two leaflets of a lipid bilayer because the polar headgroups cannot readily cross the hydrophobic membrane interior. We hypothesized that the formation of a transient hydrophilic lipidic pore in the membrane leads to diffusive translocation of negatively charged lipids through the pore to the opposite membrane leaflet. To test this hypothesis, we established a variation of the inner field compensation technique for time resolved measurements of membrane boundary potentials in asymmetric bilayer lipid membranes formed by the Montal-Mueller method. External application of electric fields across the bilayer induced transient conductive states. We observed fast transitions between these different conductance levels, reflecting opening and closing of meta-stable lipidic pores. Comparison of the capacitance minimization potential for different asymmetric membranes before and after pore formation confirmed negatively charged lipids transfer across the bilayer. We also constructed a model governing lipid flow rate based on pore analysis and lipid lateral diffusion rate. Together, our study provides a new tool to monitor loss of membrane asymmetry and our results indicate that lipid transfer can occur through lipidic pores.

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Percolation Thresholds for Diffusing Particles of Nonzero Radius: Circular Obstacles in the Two-dimensional Continuum

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Lateral diffusion in the plasma membrane is obstructed by proteins bound to the cytoskeleton. The most important parameter describing diffusion in the presence of immobile obstacles is the percolation threshold, where long-range connected paths disappear and the long-range diffusion coefficient D goes to zero. To describe obstructed diffusion, it is more accurate to find the threshold directly than to extrapolate a low-density expansion in the obstacle concentration to find the concentration at which $D = 0$. The thresholds are well-known for point diffusing particles on various lattices or the continuum. But for particles of nonzero radius, the threshold depends on the excluded area, not just the obstacle concentration. Earlier results [Saxton, Biophys J 64 (1993) 1053] for the triangular lattice showed a very rapid decrease in the threshold as the radius of the diffusing particle increases, but a lattice model gives very low resolution. The current work finds the percolation threshold for circular obstacles in the two-dimensional continuum as a function of the radius of the diffusing species. The thresholds are obtained by a Monte Carlo method based on the Voronoi diagram for the obstacles. Each Voronoi bond is by definition the path equidistant from the nearest pair of obstacles, so the separation of that pair determines whether a diffusing particle of a given diameter can traverse that bond. For point obstacles, then, one can choose a threshold corresponding to the diameter of the diffusing particle, set the conductivity of all bonds narrower than that diameter to zero and all wider bonds to one, and test for bond percolation on the resulting Voronoi diagram. The results are used to find the thresholds for lipids and for proteins of different diameters. (Supported by NIH grant GM038133)

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Effects Of Hydration On The Dynamics Of Water In Lipid Bilayer Systems: A Molecular Dynamics Study

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The properties of interlamellar water are critically important to the structure and function of biological membranes. Recent developments in femtosecond infrared spectroscopy on membrane systems at various hydration levels have opened the possibility of direct comparison between experiment and molecular dynamics simulations on the same time scales. The interpretation of experimental findings can benefit from a detailed computational study of water solvation structure and dynamics of inter-lamellar water at these hydration levels. In this presentation, we report molecular dynamics simulations of 1-palmitoyl-2-oleoyl-phosphatidylcholine POPC bilayers in the liquid-crystalline state and at three hydration levels. Simulations were performed in the canonical ensemble using the GROMACS software package. The extent to which water is influenced by the presence of membrane depends on the hydration level. We found the anisotropic diffusion constant of lipid water exhibits interesting crossover behavior as the water molecule moves from the head group region toward the bulk region. The anisotropic hydrodynamic diffusion of water is explained by structural perturbation of the water hydrogen bond network by the lipid. Radial distribution function, spatial distribution function, and power spectra of water are calculated to consolidate our interpretation. This work was partially supported by the National Science Foundation under Grant No. DGE-0221680.

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Photophysical Properties of Novel Ruthenium Metal-Ligand Complexes incorporated in Lipid Membrane Bilayers

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We have designed and synthesized novel metal-ligand complexes with amine- or acyl- reactive functional groups. These complexes have potential as luminescent probes to investigate bio-macromolecular dynamics on the submicrosecond-to-microsecond timescale. This time scale is of interest, for example, for analysis of the motions associated with large macromolecular assemblies and interactions involving membrane-bound proteins. Here we report the photophysics and structural properties of (1) the complex $[HRu(CO)(dicarboxy\text{-}bipyridyl)(PPh_3)_2]^+$ $[PF_6]^-$ conjugated to the lipid dipalmitoyl-phosphatidylethanolamine (DPPE) and (2) the complex $[(CF_3CO_2)Ru(CO)\text{-}(5aminophen)(Ph_2PC_2H_2PPh_2)]^+$ $[PF_6]^-$ conjugated to cholesterol. The conjugated complexes were incorporated in unilamellar lipid membrane vesicles to investigate the photophysical properties of these probes in the membrane environment and to evaluate the utility of these probes for investigating the physical properties of lipid membranes.

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Evaluating Gramicidin A Channel Backbone Dynamics by Molecular Dynamics and Nuclear Magnetic Resonance

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