mechanism for cholesterol trans-membrane flipping, transfer rates were measured for few cholesterol analogues with a slightly different molecular structure and thereby the geometrical and chemical compatibility with lipids. In addition, MD simulations were performed to measure the energetic and get the better understanding at molecular level for cholesterol transport. Overall, this work provides new insight in to cholesterol transport behavior in model lipid membrane.

3408-Pos Board B513

Unique Cholesterol Transport Behavior in Phosphoserine Vesicles: A Small Angle Neutron Scattering Study

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Cholesterol is critical for various cellular functions; however its excess is toxic. Cholesterol levels are maintained by various cholesterol metabolic pathways which depend critically on Intracellular cholesterol transport. Cholesterol is not homogeneously distributed in cell with 60-70% of cellular cholesterol present in plasma membrane and only 0.01-0.5% of cellular cholesterol present in endoplasmic reticulum. Any disruption in cholesterol distribution disorder has been linked to diseases such as Niemann Pick TYPE-C and Alziemers. It has been suggested that variable affinity of cholesterol for different lipids compositions could be one of the possible reason for the uneven distribution of cholesterol within cell. Traditionally the cyclodextrine or cholesterol oxidases have been used to measure the relative affinity of cholesterol for a particular lipid composition. However, the possible disruption of lipid-cholesterol interactions due to these molecules is unknown. This present study employs small angle neutron scattering to measure the cholesterol inter- and intra-membrane transport rate in model lipid vesicle without employing cholesterol tags and molecules such as cyclodextrine or cholesterol oxidase. The diffusion behavior was compared between two POPC vesicles and two POPS vesicles. Interestingly cholesterol exchange kinetics follows a non-continuous Arrhenius behavior in POPS membranes as compare to linear behavior in POPC membranes. Further cholesterol exchange kinetics was compared from POPC to POPS vesicles and POPS to POPC vesicles. Interestingly equilibrium cholesterol distribution changes with both cholesterol concentration and temperature. To our surprise we found that even a small amount of cyclodextrine can significantly shift the equilibrium distribution of cholesterol between POPS and POPC vesicles. Overall this wok provides insight in to POPS-cholesterol interaction and potential role of POPS is regulating intracellular cholesterol transport.

3409-Pos Board B514

Fluorogenic-Antioxidants: Novel Probes for Visualizing Reactive Oxygen Species in the Lipid Membranes of Live Cells

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We are pioneering the preparation of lipophilic fluorogenic antioxidant probes for the specific imaging of ROS in the membrane of live cells. Our strategy involves synthesizing a two segment receptor-reporter type free radical scavenger-fluorophore probe (an off-on fluorescent antioxidant indicator). The receptor segment in the probe mimics the structure and activity of the naturally occurring antioxidant α-tocopherol. A covalently tethered bodipy fluorophore serves the purpose of reporting, via emission enhancement, structural changes at the receptor end which result from the radical scavenging activity of the receptor.

Here we will present our most recent results involving the preparation of a second generation set of probes relying on newly synthesized bodipy dyes with improved redox properties. The new fluorogenic antioxidant probes undergo a 30 fold fluorescence enhancement upon reaction with peroxyl radicals in model lipid membranes. We will also illustrate a high-throughput fluorescence method enabled by the new probes, for the rapid determination of relative rates of free radical scavenging by α-tocopherol analogues. Rates are evaluated for tocopherol analogues with a modified lipophilic tail, when embedded in liposomes prepared from either unsaturated or saturated lipids, upon exposure to either hydrophilic or lipophilic peroxyl radicals. This work provides new insights and a quantitative understanding on the critical role of lipid diversity in modulating chemical reactions in the lipid milieu. Finally studies will be described where we utilize these new probes to image ROS in the lipid membrane of live cells.

3410-Pos Board B515

Relationships Between Bilayer Phase and Equilibration Rates of Patman and Laurdan

Hannabeth A. Franchino, John D. Bell.

Patman is a fluorescent membrane probe related to laurdan. The structural distinctions between the two probes are the lengths of the aliphatic tails (eleven carbons in laurdan and fifteen in patman) and the presence of a trimethylammonium forming a positively-charged head on patman. Studies exploring patman as a probe to detect membrane properties during apoptosis revealed that the two edges of the emission spectrum (435 and 500 nm) stabilize at different rates as the probe binds to the cell membrane. To test whether these differences represent dissimilarities in probe binding to ordered and disordered domains, experiments were conducted to monitor patman equilibration with bilayers composed of various mixtures of saturated and unsaturated phosphatidylcholines at temperatures above, at, and below the main thermotropic phase transition. In general, patman equilibrated more rapidly with bilayers in the liquid disordered phase than in the solid ordered phase. With solid phase membranes, the fluorescence stabilized faster at 435 nm than at 500 nm. Similar yet more subtle results occurred in the lipid disordered phase. In contrast, the situation was reversed at the phase transition temperature; equilibration was faster at 500 nm than at 435 nm. To determine whether these results reflected specific properties of patman, the experiments were repeated with laurdan, and several distinctions were observed. First, equilibration with solid phase lipids was faster than for patman and not different from equilibration with the fluid phase. Second, differences in rates between the two wavelengths were less than with patman for solid phase membranes but greater than with patman for melted bilayers. Third, at the phase transition temperature, equilibration rates favored 435 nm over 500 nm, the opposite of the result obtained with patman. Computer simulations were used to assist with interpretation of these results.

3411-Pos Board B516

Fluorescent Correlation Spectroscopy and Raster Image Correlation Spectroscopy as a Tool to Measure Diffusion in the Human Epidermis

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Structural and dynamical characterization of skin tissue is vital for understanding the behavior of healthy and diseased skin tissue. Our objective is to develop protocols to measure the local diffusion of substances with different physical properties (for example amphiphilic or hydrophilic drugs) in the epidermis and dermis using fluorescent lipid analogs and hydrophobic dyes as model systems. As an example we use fluorescent labeled liposomes with a lipophilic dye in the bilayer and a hydrophilic dye inside. Using two color FCS (Fluorescent Correlation Spectroscopy) and two color RICS (Raster image correlation spectroscopy) we determine the diffusion and if intact liposomes penetrate the epidermis or if the burst before penetration. The experiments were preformed on a custom build multi-photon microscope[1]. Finally advantages and disadvantages of the different techniques for measuring diffusion in skin tissue are compared and discussed.

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3412-Pos Board B517

Concentration Dependent Membrane Anchor Colocalization Study by Fluorescence Cross-Correlation Spectroscopy in Live Cells

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Membrane anchors such as protein lipidations and glypiations have been proposed to play essential roles in the sorting and organization of plasma membrane-associated proteins, especially those involved in cell signaling. Here, we investigate the concentration dependence and variability of anchor colocalization in live cells by transfecting various cell types with pairs of fusion proteins created by replacing all but short tails of natively lipidated proteins with either red or green fluorescent proteins. These fusion proteins remove any native protein-protein interactions while fluorescently tagging membrane anchors in live cells. To observe sub-cellular organization, we use Fluorescence Cross-Correlation Spectroscopy (FCCS) to quantify the dynamic colocalization between green- and red-labeled anchors. FCCS allows observations of dynamic colocalization in live cells at a greater range of separation distances than is allowed by FRET, and because it is a dynamic measurement FCCS avoids ambiguous or false positive colocalization that can result from static studies. Fusion protein expression level, as determined by overall intensity of cell fluorescence, naturally varies in a population of transiently transfected cells. Using this to our advantage, we are able to observe cells within a wide range of protein expression and explore trends between concentration and fusion protein colocalization. We also analyze variation in the amount of colocalization and observe a difference between the variability from cell-to-cell and the variability from spot-to-spot within one cell across several anchor types and different cell lines.