general, mechanisms involved in particle release from platelets and erythrocytes appeared relevant to lymphocytes.

418-Pos Board B204

Effects of Cations on Phase Properties of Dipalmitoylphosphatidylcholine Assessed by Laurdan Fluorescence

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Two mechanisms have been proposed to account for the reduction in membrane fluidity observed in the presence of high concentrations of certain salts: direct binding to phospholipid molecules and dehydration of the membrane. To address these proposals experimentally, we prepared dipalmitoylphosphatidylcholine liposomes with laurdan, which is sensitive to the presence and mobility of water molecules in the bilayer. Laurdan emission spectra and steady-state anisotropy were then acquired simultaneously at multiple temperatures below and above the main phase transition. Calcium ions (1 M) raised the transition temperature by 8.5 K with minimal effects on the apparent transition cooperativity and ΔH. Moreover, laurdan spectra (quantified by generalized polarization, GP) were blue-shifted at all temperatures (by 0.05-0.1 GP units) confirming modest dehydration of the membrane. Anisotropy reflected only the effects on the phase transition with no alterations to values at the temperature endpoints suggesting that the mobility of laurdan was unaltered by calcium. In contrast, sodium ions produced little change to the transition temperature, but reduced both the apparent cooperativity and ΔH (by ~25%). The laurdan GP was identical to control samples in the lipid gel phase, but elevated by more than 0.1 GP units at temperatures above the phase transition, suggesting that dehydration by the salt only occurred in the fluid phase. Surprisingly, anisotropy was lowered at all temperatures by sodium, reflecting greater mobility of laurdan notwithstanding the negative impact of the ion on overall membrane fluidity. Effects of potassium on the phase transition were similar to those of sodium, but no change in either GP or anisotropy were observed at the endpoint temperatures. These results argue that the mechanism of salt effects on membrane properties is more complex and ion-specific than previously hypothesized.

419-Pos Board B205

2H Solid-State NMR Studies of the Antimicrobial Peptide MSI-78 Interacting with the Membranes of Whole Escherichia Coli

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Antimicrobial peptides (AMPs) are ubiquitous molecules that can display antimicrobial activity against bacteria, viruses, protozoa and various other pathogens. A key aspect of AMP activity is their interactions with biological membranes. Solid-state NMR spectroscopy has thus been an important tool for their study. Previous experiments, on model membrane systems, have elucidated important aspects of AMP mechanism. However, the extent to which actual in vivo AMP activity can be understood from model studies is necessarily limited. Peptide-membrane interactions under physiological conditions are presumably influenced by additional factors such as: interactions with lipopolysaccharides, the presence of membrane proteins, membrane compositional heterogeneity, lipid domains, etc. In order to bridge the gap between the NMR studies of AMPs using model membranes and the AMP-membrane interactions occurring in intact cells, we have designed a procedure to incorporate high levels of 2H-NMR labels, specifically into the cell membrane, by creating a novel strain of E. coli: LA8. Using this strain we are able to reproducibly quantify the effects of the AMP MSI-78 on lipid chain order in bacterial membranes. Treatment with MSI-78 led to an increase in the disorder of the bacterial membrane. This was observed by the decrease in the average order parameter and by the increase in intensity at the lower frequencies. The peptide:lipid ratios needed to observe MSI-78's effects on acyl chain order in the intact cells falls between the ratios required to observe effects in NMR studies of model lipid systems and the ratios required to observe inhibition of cell growth in biological assays.

420-Pos Board B206

Investigation of the Role of Cholesterol Superlattice in Release Kinetics of Drugs from Stealth Liposomes

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The development of liposomal drug formulations to combat diseases such as cancer is a growing field of study, with over twenty liposomal chemotherapy drugs currently on the market or undergoing clinical trials. We have previously demonstrated that cholesterol, often employed as a membrane stabilizing agent in liposomal drugs, plays a delicate and critical role in the release kinetics of the chemotherapy drug combretastatin A4 disodium phosphate (CA4P) in accordance with the principles of sterol superlattice. Here, we investigate the impact of membrane cholesterol content on the release of the same drug from ''stealth'' liposomes, which possess a polyethylene glycol (PEG) coating that renders them nearly invisible to the immune system and allows more efficient targeted drug delivery. Samples of stealth liposome-encapsulated CA4P were prepared in which the cholesterol content differed little (0.4 mole%) between tubes in order to assess the effect of cholesterol content on a fine scale. First, we used a fluorescence assay which exploits the intrinsic fluorescence of CA4P to determine its release kinetics from liposomes in aqueous solution. Next, a cytotoxicity assay was employed to determine the effect of the various liposomal drug formulations on cancer cells in vitro. Finally, the data were correlated with the predictions of the theory of sterol superlattice and compared to previous experimental results.

421-Pos Board B207

Study of the Interactions Between Model Membranes and a Truncated Hemoglobin (trHbN) by NMR and Infrared Spectroscopy

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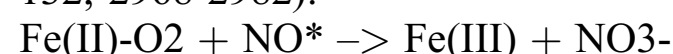
Approximately one third of the world population is infected by the pathogenic bacterium Mycobacterium tuberculosis. A key to the resilience of M. tuberculosis resides in part in its capacity to enter a latent state where it can resist different oxygen and nitrogen oxidative species such as hydrogen peroxide, nitric oxide and peroxynitrite produced by the infected macrophages. One protein responsible for that is trHbN, a truncated hemoglobin, that detoxifies nitric oxide from the cellular environment. It is thought that the protein heme achieves that through this mechanism (Mishra et al, J. Am Chem. Soc., 132, 2968-2982):



Fe(II)-O2 + NO* –> Fe(III) + NO3-

The importance of studying this protein lies in the fact that we now have to deal with new antibiotic-resistant strains. Therefore, we have investigated the trHbN orientation and conformation in different lipid model membranes. We also have studied the effect of the protein on these membranes. FTIR was used to observe changes in the conformational order of the lipids in the presence of the protein and the protein secondary structure. Furthermore, solid-state NMR provided information on the membrane conformation and on the protein orientation. These studies were performed in pure lipids, and also in a mixture of two different lipids (TOCL and DOPE) which was optimized to achieve a composition similar to that of the bacterial membrane.

422-Pos Board B208

Using Super-Resolution Fluorescence Localization Imaging to Probe Raft Hetergeneity in Fixed and Live Cells

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We are using super-resolution fluorescence localization imaging to probe the organization and mobility of 'raft' and 'non-raft' markers in the plasma membranes of mammalian cells. Our goal is to experimentally test our recent predictions regarding protein organization and mobility in membranes containing critical fluctuations that are coupled to cortical cytoskeleton (1). Our raft constructs include the transmembrane domain of linker of activated T cells (LAT-TM) the transmembrane domain of influenza hemaglutinin protein (HA-TM), GPI-linked proteins, and cholera toxin B subunit bound to the ganglioside GM1. Our non-raft markers include palmitoyl-null mutants of LAT-TM and HA-TM constructs, and the lipid probe $DiIC_{12}$. We are probing the organization and mobility of the above markers either through conjugated photoactivatable (PA) fluorescent proteins (PALM), or through the reversible blinking of organic fluorophores (STORM). By measuring autocorrelations and cross-correlations from one and two color fixed cell images, we quantitatively probe the nano-scale organization of components and can compare our findings to our recent predictions. We quantitatively probe the diffusion and confinement of membrane components by measuring the mean