bacterium and the precise width of the bacterium in the physiological environment. The results were compared with similar results obtained from dead bacteria. High-resolution optical microscopy was employed to monitor the viability of the bacteria under study before and after the stabbings. The evidence suggests that bacteria are still alive after multiple puncturings! The results are tentatively explained in terms of self-repair of the lipid bilayers and of the peptidoglycan layer of *S.* Typhimurium against multiple puncturing events exerted by an AFM tip.

2325-Pos Board B295

Activation Dependent Organization of T Cell Membranes: A FCCS Study

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While the heterogeneity of the plasma membrane of eukaryotic cells is by now a well-established fact, the precise architecture of this important cellular structure is still seriously debated. Here, we focus on the role of specific lipid anchor motifs in the organization of T-cell plasma membranes into distinct domains of particular composition. To that end we generated a combinatorial library of protein constructs by fusing different lipid-modification sites of lipid anchored proteins with one of two fluorescent proteins. Two of these constructs that encode for either myristilation, palmytilation, geranylation or glycosylphosphatidylinositol (GPI) elaboration and are labeled with either enhanced green fluorescent protein (EGFP) or monomeric Cherry fluorescence protein were co-expressed in each cell. We used dual color fluorescence cross-correlation spectroscopy (FCCS) to exploit co-movement of the same or different lipid anchors as a signature of spatial cluster formation, thereby circumventing the limitations of direct imaging of nano-meter sized membrane structures. Our comparative FCCS studies on membranes of whole T cells and plasma membrane sheets show that in living T cells most anchors only co-localize with themselves, while different anchors move independently from each other. This suggests that the plasma membrane is composed of a variety of different domains (each with specific protein content) and that the lipid anchor structure plays a key role in the specific recruitment of proteins into their target domains. However, in equilibrated membrane sheets, some of the selective aggregation is lost. We also find significant differences in the degree of aggregation between activated and non-activated T cells and their sheets. Furthermore, cholesterol depletion and actin-drug experiments indicate that both actin as well as cholesterol is involved in the dynamic nano- and micrometer scale organization of the T cell plasma membrane.

2326-Pos Board B296

Detection of Lipid Domains in Model and Plasma Membranes by Fluorescence Lifetime Imaging Microscopy of Fluorescent Lipid Analogues

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The presence of lipid domains in cellular membranes and their characteristic features are still an issue of dividing discussion. Several recent studies implicate that lipid domains in plasma membranes of mammalian cells are short lived and in the submicron range. To unravel the lateral heterogeneity of cellular membranes, in particular of mammalian plasma membranes, at this scale various techniques of fluorescence spectroscopy and microscopy have been applied. Measuring fluorescence lifetime of appropriate lipid analogues is a proper approach to detect domains with such properties. Here, the sensitivity of the fluorescence lifetime of 1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-sn-glycero-3-phospholipid (C6-NBD-phospholipid) analogues has been employed to characterize lipid domains in Giant Unilamellar Vesicles (GUVs) and the plasma membrane of mammalian cells by Fluorescence Lifetime Imaging (FLIM). For GUVs forming microscopically visible lipid domains the fluorescence lifetime in the liquid disordered (ld) and the liquid ordered (lo) phase was clearly distinct being about 7 ns and 11 ns, respectively. Lifetimes were not sensitive to variation of cholesterol concentration of domain forming GUVs indicating that the lipid composition and physical properties of those lipid domains are well defined entities. Even the existence of submicroscopic domains can be detected by FLIM. Application of this approach to mammalian cells revealed that while the fluorescence lifetime is sensitive to the composition of the plasma membrane, distinct lipid domains as found for GUVs were not detected. A broad distribution of the long lifetime was found for C6-NBD-PC inserted in the plasma membrane of these cells centred around 11 ns. However, FLIM studies on lipid domain forming giant vesicles derived from the plasma membrane of HeLa-cells rather support a recent hypothesis that an ensemble of lipid domains being of submicroscopic size exist in the plasma membrane.

2327-Pos Board B297

Probing Membrane Domains and Diffusion Barriers in Live Sperm Cells Using Fluorescent Particle Tracking

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Polarized cells, e.g. neurones and spermatozoa, characteristically contain compositionally distinct domains in their plasma membranes that are commensurate with specialized function. Elucidating the mechanisms that generate and maintain these heterogeneities is fundamental to understanding many of the processes involved in cell differentiation. Spermatozoa are excellent models for studying membrane compartmentalization as several distinct domains are present on the surface of the head [1,2]. In this work we probe the nature of the barriers that separate these regions by analyzing the trajectories of individual fluorophore-labelled lipids and proteins as they diffuse within and between domains.
The probes used were wheat germ (WGA) lectin, DOPE, $DiIC_{16}$ and cholera toxin β-subunit (CTXB) and were delivered either as single molecules from a nanopipette [2] or from suspension. Results showed that single protein and lipid molecules exchanged freely between all domains on the head plasma membrane. Conversely, particles of $DiIC_{16}$ and clusters of CTXB cross-linked GM1 gangliosides, ranging in size from 0.5 to 2.0 microns, showed confinement and were unable to traverse domain boundaries. We hypothesise that a mass filter is present within the membrane that is permissive to single molecules but not multimolecular complexes. Relocation and assembly of these molecules and complexes on the sperm head in response to external stimuli is likely to be important in the developmental processes that lead to successful fertilization.
[1] James *et al.* (2004) *Journal of Cell Science* **117** 6485-6495.
[2] Bruckbauer *et al.* (2007) *Biophys. J.* **93** 3120-3131.

2328-Pos Board B298

Biophysical, Structural and Compositional characterization at the molecular level of Native Pulmonary Surfactant Membranes directly isolated from mice Wild-type and Knocked-out Protein D Bronco-alveolar Lavage Fluid

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Pulmonary surfactant is a surface active material composed of lipids and proteins produced by type II pneumocytes cells in the alveoli. This tension-active material forms a unique interface separating gas and liquids at the alveolar cell surface, reduce surface tension close to 0mN/m and maintains lung volumes and alveolar homeostasis at the end of the expiration. Abnormalities of surfactant in the immature lung or in the acutely inflamed mature lung are related to several illnesses. There are four pulmonary surfactant proteins (SP-A, -B, -C and -D). SP-A and -D have a very important role in the immunological response against pathogens. The particular lipid composition of the lung surfactant suggests that native surfactant mono- and bi-layer-based structures could exhibit lateral segregation phenomena at physiological temperatures. The principles underlying the interfacial film and membrane-base organization are not well defined. Therefore, a deep study in the correlation among surfactant composition, structure and biophysical function is needed. The present work tries to get advantage of the combination of different biochemical and biophysical techniques applied to Native Pulmonary Surfactant Membranes directly isolated from wild-type and KO protein-D mice bronco-alveolar lavage fluid (BALF). Both mono- and bi-layers show the presence of different structural arrangements, which could indicate a phase lateral coexistence. A closer look at the lipid composition reveals several potential lipidic species which might be playing a role in the segregation phenomena in addition to SP-D. A detailed characterization and correlation with surfactant biophysical functional properties, as well as with particular molecular species is currently being performed. This experimental approach is extremely powerful to correlate the structural, biochemical, and biophysical properties of any compositionally complex material.

2329-Pos Board B299

Studying the In Vivo Behavior of the Vesosome

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An optimal drug delivery vehicle should circulate in the body long enough to reach the site of illness or disease and also localize itself at the desired site

to consequently deliver its contents at a rate appropriate for maximum therapeutic benefit. It should also possess a large drug loading capacity and retain its contents over the course of treatment. While liposomal systems have experienced success with extending circulation, content retention and controlled release remain problematic. The vesosome - a large lipid bilayer enclosing many smaller liposomes - is the most suitable candidate for addressing these issues. The external lipid bilayer offers a second barrier of protection for interior components and also serves as the anchor for active targeting components. Furthermore, internal compartmentalization permits customization of separate environments for multiple therapeutics and release triggers, highlighting the vesosome's potential as a single site, single dose, multiple component drug treatment.
To assess the viability of the vesosome as a drug carrier, its in vivo lifetime and biodistribution was examined in live animals. Our work examines how these properties are affected by lipid composition and the addition of other functional components, including ones for controlled release and active targeting.

2330-Pos Board B300
Hyperglycemia Promotes Membrane Cholesterol Crystalline Domain Formation Through Lipid Peroxidation: Inhibition with Atorvastatin Metabolite
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Insulin resistance and poor glycemic control contribute to atherogenesis through the chemical and structural modification of cell membrane lipids. The direct contribution of glucose to these membrane alterations, however, is not well understood. In this study, small angle x-ray diffraction and spectrophotometry were used to examine the autoxidative effects of glucose on lipid oxidation and structural organization in model membranes comprised of dilinoleoylphosphatidylcholine (DLPC) and cholesterol. Membranes were prepared at cholesterol-to-phospholipid (C/P) mole ratios ranging from 0.2 to 0.8 in order to model physiologic and hyperlipidemic conditions. Changes in membrane lipid organization and unit cell periodicity (*d*-space) were correlated with lipid hydroperoxide (LOOH) concentration measured at 24 hr intervals. The effects of glucose on lipid peroxidation were more pronounced at elevated levels of membrane cholesterol, with LOOH levels 20% higher at 0.8 C/P than at 0.2 C/P. At 0.6 C/P, glucose treatment resulted in a concentration-dependent increase in LOOH formation as compared to control. These changes corresponded to a reduction in membrane bilayer width (51 Å to 49 Å) and the progressive formation of highly-ordered cholesterol crystalline domains (*d*-space value of 34 Å). Treatment with atorvastatin hydroxy metabolite, a statin with scavenger antioxidant properties, inhibited the membrane-altering effects of hyperglycemia in a dose-dependent manner, even at elevated cholesterol levels. These data demonstrate that glucose directly stimulates lipid peroxidation and subsequent changes in membrane structure, including the formation of immiscible cholesterol crystalline domains. Insights from this study may also serve as a model for better understanding the membrane structural changes associated with diabetes and related complications.

2331-Pos Board B301
Characterization of a New Biomimetic Multilayer System for Biomembrane Interaction Studies
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Layer-by-layer (LbL) deposition methods were shown to be especially suitable for polyelectrolyte multilayers (PEM) as stable and functional supports for various bio-mimetic systems. Here, results from an investigation of the interaction between chitosan/heparin PEM films and small unilamellar lipid vesicles (SUV) are presented. The membranes were composed of a mixture of zwitterionic POPC and its cationic counterpart E-POPC thus having a positive surface charge density. Surface Plasmon Resonance (SPR) was applied to continuously monitor the self-assembly process of physisorption of subsequent PE layers and to report the deposition efficiency and dynamics. A terminating lipid bilayer was successfully deposited on top of the PEM films, both with chitosan and heparin as uppermost PE layer. The lipid layer could be totally removed by detergent application without damage to its PEM cushion. The PE film itself was studied by atomic force microscopy (AFM) in its dry and also in its fully hydrated state. The integrity and homogeneity of the terminal lipid bilayer on its PEM cushions was also visualized with the AFM technique. Currently, neutron reflectivity is being applied to further investigate of the multi-layer structure of the composite film and its hydration. Experiments with confocal microscopy and applying SFS (sum frequency spectroscopy) are under preparation.

Interfacial Protein-Lipid Interactions I

2332-Pos Board B302
Interaction of Tea Catechin (−)-Epigallocatechin Gallate with Lipid Bilayers
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A major component of green tea extracts, catechin (-)-Epigallocatechin gallate (EGCg) has been reported to be biological active and interacting with membranes. A recent paper reported drastic effects of EGCg on giant unilamellar vesicles (GUVs). In particular, EGCg above 30 μM caused GUVs to burst. Here we investigated the effect of EGCg on single GUVs at lower concentrations, believing that its molecular mechanism would be more clearly revealed. We used the micropipette aspiration method, by which the changes of surface area and volume of a GUV could be measured as a result of interaction with EGCg. We also used X-ray diffraction to measure the membrane thinning effect by EGCg. To understand the property of EGCg, we compared its effect with other membrane-active molecules, including pore-forming peptide magainin, the turmeri (curry) extract curcumin, and detergent Triton X100. We found the effect of EGCg somewhat unique. Although EGCg readily binds to lipid bilayers, its membrane area expansion effect is one order of magnitude smaller than curcumin. EGCg also solubilizes lipid molecules from lipid bilayer without forming pores, but its effect is different from Triton X100.

2333-Pos Board B303
How Do Electrostatic Interactions Affect The Behavior Of Transmembrane Peptides?
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It has been shown that changes in physical properties of the membrane, such as surface charge or fluidity, affect the activity of embedded proteins. This is likely related to the presence of polar and/or aromatic residues that are often observed in the interfacial regions of those proteins. There mutation often results in modification of their activity. This raises the question: how do those polar and/or aromatic residues affect the orientation and dynamic behavior of transmembrane segments of proteins, thus affecting protein activity?
Here we try to understand how electrostatic interactions affect transmembrane segments by use of simplified model systems consisting of KALP and WALP peptides. These peptides are composed of alternating alanine and leucine stretches flanked with lysines or tryptophans residues respectively. The peptides are embedded in vesicles containing Zwitterionic (DMPC), negatively charged (DMPG, DMPS, DMPA), or positively charged (DMTAP) lipids. The samples are then analyzed with 2H or 14N and 31P wide line solid state NMR methods.
The results show that lipid composition affects transmembrane peptides in different ways depending on whether they are flanked with lysines or tryptophans. The results highlight the different properties of salt-bridge interactions and cation-pi interactions, and their possible implications in membrane protein activity.

2334-Pos Board B304
Orientation Of A Transmembrane Peptide Under Positive Mismatch By Computer Simulations
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This work deals with the orientation of a transmembrane model peptide (WALP23) under positive mismatch, assessed by atomistic molecular dynamics simulations. Emphasis was given to link our results to deuterium solid state NMR data of the same system under the same mismatch conditions. So far, small tilt angles were extracted from the experimental quadrupolar splittings using a geometric analysis, called the GALA method. The backcalculation of these NMR quadrupolar splittings from our simulations showed a good fit with experimental data only if several hundred of nanoseconds trajectories were considered. Some coarse-grained simulations allowed us to reach the NMR time scale (a few microseconds) and led to the same observation. For both types of simulation we found that some averaging effects may affect the interpretation of NMR data, and thus larger tilt angles than previously estimated are likely to occur.