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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