

2582-Pos Board B274**The Effect of Ectoins on the Structural Organization of the Tear Fluid Monolayer**Hans Joachim Galla^{1,2}, Mridula Dwivedi¹.¹Biochemistry, Westfälische Wilhelms Universität Münster, Münster, Germany, ²Chemistry and Pharmacy, Institute for Biochemistry, Muenster, Germany.

We investigate the influence of Ectoin on the structural organization of the natural and artificial tear fluid lipid layers (ATFLL) using surface activity analysis and topographical studies. The natural meibomian lipids exhibit a continuous pressure-area isotherm without any phase transitions. In the presence of ectoin, the isotherm is expanded towards higher area per molecule implying decreased interaction between the lipid molecules. The AFM scans show presence of fiber like structures in the natural meibomian lipid film. In the presence of ectoine, droplet-like structures are observed which are hypothesized to be triacyl glycerols excluded from the lipid film. ATFLL illustrate the fluidizing effect of ectoine on the lipid films where the pressure-area isotherms are expanded in the presence of ectoin. With the addition of a triacyl glycerol to the mixture of DPPC and Chol-Palmitate, we observed the formation of similar drop-like structures in the presence of ectoine as in the case of natural meibomian lipid films. Consequently, the hypothesis explaining the exclusion of tri/di acyl glycerol from the meibomian lipid film in the presence of ectoine in the subphase is confirmed which lead us to a model describing the fluidizing effect of ectoine on meibomian lipid films.

2583-Pos Board B275**Lipid Order Investigations Combined with Generalized Polarization Provide Deeper Insights into Plasma Membrane Architecture of Live Cells**

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The complexity of cell plasma membrane architecture is governed by biomolecular interactions, heterogeneity and complex sub-micrometric size domains. Even though there is no doubt about the existence of liquid-ordered and liquid-disordered phases in the model membranes, direct observations in live cells remain delicate due to their dynamic nature and sub-resolution size.

High resolution optical microscopy techniques have helped the understanding of lipid organization and their contribution to biological functions. Among them, environmentally sensitive lipid probes (such as Laurdan or derivatives of ANEP) reveal lipid packing information (in particular fluidity governed by local polarity) at the nanometer scale, thanks to the bathochromic shift in their fluorescence emission spectrum as the membrane undergoes phase transition from gel to fluid [1]. The associated spectral ratiometric imaging is called generalized polarization (GP).

In this work, we combine GP imaging with orientational investigations on such lipid probes, in order to probe, in a complementary approach, both local lipid packing (which reports a molecular scale information) and lipid order (which report a mesoscopic scale information). The orientational order of lipid probes, measured by fluorescence angle-resolved linear dichroism microscopy (FARLDM), has been previously shown to be highly sensitive to local membrane morphological changes (driven by cytoskeleton alterations) and cholesterol depletion [2].

Implementing a combined GP-FARLDM technique, we show that new information can be gained on lipid interactions at different scales in the cell plasma membrane. We show in particular that liquid-ordered and liquid-disordered phases exhibit distinct morphological behaviors, and explore the capacities of different lipid probes to report information on the membrane architecture at the nanoscale.

References

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2584-Pos Board B276**Spin Labels Detect the Coexistence of Two Lipid Domains Along the Anomalous Gel-Fluid Transition of Anionic Dmpg Bilayers**

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Vesicles of the acidic lipid dimyristoyl phosphatidylglycerol (DMPG), at physiological conditions, show a highly cooperative gel-fluid transition, around 23°C. However, at low ionic strength, DMPG bilayers display a peculiar thermo-structural behavior, presenting a broad gel-fluid transition, between 18°C and 35°C. Several interesting properties of this "transition region" led to different models for DMPG dispersion at low ionic strength. Here, we use computer simulations (1) of the electron spin resonance signal of spin labels

incorporated into DMPG vesicles to evaluate the structure of the vesicles along the gel-fluid transition. At temperatures below and above the phase transition, in the gel and fluid phases of the bilayer, only one lipid population can be detected. But along the phase transition region, two lipid populations coexist: one very mobile, even more fluid than lipids in the bilayer fluid phase, and another one more rigid and organized, typical of lipids in the gel phase. The more mobile population appears at ca. 18°C and disappears by the end of the phase transition. In accord with previous works (2-4), we propose that this highly mobile lipid population is associated with the formation of pores at the bilayer, and these mobile lipids are located at the edges of the pores. Hence, spin labels can monitor the increase and decrease of bilayer perforations along the membrane phase transition.

Financially supported by USP, FAPESP, CNPq.

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2585-Pos Board B277**Protein Partitioning in Liquid-Ordered (LO) / Liquid-Disordered (LD) Domains Depends on Lipid Composition and Protein Shape**Benjamin Kollmitzer¹, Peter Heftberger¹, Michael Rappolt², George Khelashvili³, Daniel Harries⁴, Georg Pabst¹.

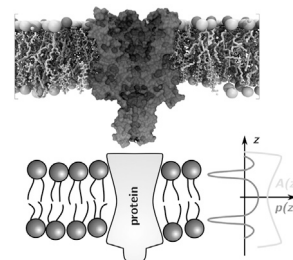
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The lack of transmembrane proteins partitioned in the current lipid-only models for membrane rafts, i.e. Lo phases, calls for close scrutiny of raft mimetics. Using small angle X-ray scattering (SAXS) and molecular dynamic simulations (MD), we determined structural and elastic parameters (spontaneous curvature, bending rigidity, Gaussian curvature modulus) for coexisting Lo/Ld domains in ternary mixtures of dioleoylphosphatidylcholine/dipalmitoylphosphatidylcholine/cholesterol (DOPC/DPPC/Chol) and dioleoylphosphatidylcholine/distearoylphosphatidylcholine/cholesterol (DOPC/DSPC/Chol) [1,2]. Substituting these values into theoretical calculations yields the energy penalty upon insertion of transmembrane proteins into Lo and Ld phases, and consequently the preferred partitioning in one of these domains. We discuss our findings for different geometric protein shapes.

References

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This work is supported by the Austrian Science Funds FWF, Project No. P24459.

**2586-Pos Board B278****Perturbation of Plasma Membrane Physical Properties by Endogenous and Exogenous Mediators Affects Cell Function**

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In mammalian plasma membranes (PM), ordered, lipid-driven lateral domains - known as lipid rafts - have been implicated as central regulators of a broad range of cell functions and pathophysiological processes. Recent observations suggest these domains comprise a major fraction of mammalian plasma membranes, so rather than isolated islands of distinct composition, they may be fundamental to PM structure and function. However, direct demonstrations of lipid raft involvement in specific cell functions remain elusive because of artifact-prone, indirect, and poorly characterized experimental paradigms for raft perturbation. Giant Plasma Membrane Vesicles (GPMVs) are isolated PMs that separate into coexisting raft and non-raft phases in a temperature dependent manner, with the miscibility transition temperature providing a quantitative estimate of the stability of raft domains in live cells. We have evaluated a number of endogenous and exogenous mediators of the plasma membrane phenotype - defined as the fluidity of PM and the stability of lipid raft domains - and measured the effects of these perturbations on cell function. Cell autonomously, we find that differentiation of Mesenchymal Stem Cells (MSC) causes rapid divergence of both aspects of the PM phenotype. Inversely, perturbation of PM physical properties by ω -3 poly-unsaturated fatty acids