the wild-type protein, face the apolar environment of the lipid bilayer. We then measured the thermodynamic stability of the wild-type protein and of each of the sequence variants by chemical denaturation. We only made these measurements when the proteins appeared to be at reversible equilibrium between folded and denatured states. We also characterized the folded states of each protein by fluorescence spectroscopy and by a functional assay. Those characterizations revealed additional information about how the lipid bilayers may accommodate an arginine.

1801-Pos Board B645

Chain Length Effect on the Association of Fluorescent amphiphiles with lipid bilayer membranes

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The lack of quantitative, kinetic and thermodynamic knowledge regarding the interaction of amphiphiles with lipid bilayers, and the biological and pharmacological relevance of this subject, prompted our group towards a detailed study of those processes [1, 2]. Here we present a detailed study of the interaction of two homologous series of fluorescent amphiphiles (containing one or two acyl chains with different lengths) with a membrane in the liquid disordered phase (POPC). The kinetic rate constants for insertion, desorption, and the corresponding equilibrium partition constants, were obtained. The study was performed as a function of temperature, and the thermodynamic parameters were also obtained.

One of the homologous series studied is a phospholipid labeled with the fluorescent group 7-nitrobenzo-2-oxa-1,3-diazol-4-yl (NBD) in the polar head group and with different lengths of the two acyl chains (NBD-diC_nPE; with n=6, 8, 10, 12 or 14). The other homologous series consists of fatty amines labeled with NBD in the amine group an different *acyl* chain lengths (NBD-C_n; with n=8, 10, 12 or 16).

In contrast to the expectation based on the current model for the transition state in the insertion/desorption process [3, 4], we found a strong dependence between the rate of insertion and the acyl chain length, for both homologous series. The interpretation and implications of the results obtained are discussed.

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1802-Pos Board B646

Effect Of The Acyl Chain Length On The Translocation Rate Of Amphiphilic Molecules In Liquid Disordered And Liquid Ordered Lipid Bilayers

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Passive transport across cell membrane is a significant route for the permeation of xenobiotics through tight epithelia, such as the vascular endothelium that constitutes the Blood Brain Barrier. One of the most important processes for permeation is transmembrane translocation, which depends on the structure of the permeating molecule and on the properties of the lipid bilayer.

In this work we report on the translocation of two homologous series of fluorescent amphiphiles between the two leaflets of lipid bilayers, in the liquid disordered phase (POPC) and in the liquid ordered phase (SpM:Chol 6:4), using established methods [1]. Both series are labeled with the probe 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) in the polar portion and have acyl chains of different length. One of the series is a phospholipid derivative (NBD-diCnPE; n=6,10 or 14) and the other is a fatty amine (NBD-Cn; n=8, 10, 12, 14 or 16). Along these homologous series, the hydrophilic group is maintained and the hydrophilic/hydrophobic ratio is changed *via* the length of the *acyl* chain. The work was done at different temperatures and the thermodynamic parameters were obtained.

For the fatty amine homologous series, the translocation rate constants recovered show a strong dependence on the length of the *acyl* chain for both lipid phases, being very fast for NBD-C8 and almost 3 orders of magnitude slower for the two longer *acyl* chains. A different behavior was found for the phospholipid homologous series, where the translocation was essentially independent on the *acyl* chain length, showing that for this series the solubilization of the polar head group in the center of the bilayer is the higher energetic barrier in the translocation process.

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1803-Pos Board B647

Unraveling the Role of Protein-Proteins Interactions of Annexin at the Membrane

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Protein-membrane interactions are a vital mechanism of propagating signals both across the membrane and between cells. One method of signal propagation is the formation of lipid microdomains that allow the preferential clustering of specific lipid types and proteins. To address this type of signal propagation, we investigated how lipid microdomains form in response to annexin binding to model membranes. Annexins bind to negatively charged (e.g., phosphatidylserine [PS]) membranes in a calcium-dependent manner and lead to the formation of PS-enriched microdomains in supported planar bilayers. Two distinct mechanisms of signal propagation via protein-lipid binding are addressed. First, we hypothesize that proteins can transmit binding information via the ordering of the lipid acyl chains upon binding. Alternatively, we predict that when a protein binds a specific lipid preferentially, protein-protein interactions are enhanced on a membrane surface. The role of lipid acyl chain ordering and protein-protein interactions as distinct mechanisms of signal propagation through lipid binding will be illustrated.

1804-Pos Board B648

Artificial Phospholipid Bilayers On Nano-patterned Gold Surfaces For Biosensing

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As cell surface mimics, supported lipid bilayers are suitable as functional overlayers that enable the study of binding interactions that occur at cell surfaces. These interactions are relevant to cell-cell interactions, and pharmacological applications. Their use however, is limited by the types of surfaces they can reliably be assembled on. We demonstrate the assembly of artificial phospholipid bilayers on gold substrates patterned with a regular array of nano-holes. The lipid layers are characterized by imaging and force indentation using an atomic force microscope. We also demonstrate a biosensor that combines nano-hole arrays, and lipid bilayers.

1805-Pos Board B649

Fission Of Lipid Nanotube By Osmotic Pressure

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Formation of new membrane compartments, such as transport vesicles in cells, is finalized by scission of the membrane connection between the vesicle and the parent membrane. To avoid leakage of the vesicle contents, fission has to pass through so called hemifission state, where inner monolayer of membrane neck self-merge while outer preserves its continuity. Creation of hemifission is coupled to generation of high membrane curvature by specialized protein machinery. To reveal the intrinsic behavior of lipid bilayer in this process we studied protein-free fission of membrane nanotubes (NT) subjected to osmotic stress. As expected, lowering of the osmolarity of the external solution caused NT expansion while increasing of the osmolarity produced NT narrowing. We found that osmotic pressure could squeeze NT to a critical radius where non-leaky fission occurred spontaneously. Furthermore, when we progressively increased the amount of cholesterol in the NT membrane to augment its rigidity, the value of the critical radius remained unchanged (corresponding to the lumenal radius of approximately 2 nm). Thus we conclude that membrane rearrangements leading to non-leaky membrane fission can be initiated by a critical narrowing of the membrane tubule.

1806-Pos Board B650

Measurement Of Mechanical Parameters Of Lipid Bilayer Form The Deformation Of Membrane Nanotube In Electric Field

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The effect of a longitudinal electric field on the shape of a lipid nanotube formed in an electrolyte solution is considered experimentally and theoretically. Application of a moderate (50-250mV) potential difference between two ends of the nanotube caused the tube expansion so that its shape deflected from the initial cylindrical to the parabolic one. The magnitude of this deviation depends on 1) the potential difference applied, 2) initial lateral tension and 3) bending modulus of the nanotube membrane. This deviation can be quantified as an effective radius of the nanotube determined by the mechanical parameters of the nanotube membrane and the magnitude of the applied electrical field. From the dependence of this radius on the potential difference the values of

both lateral tension and bending rigidity of the nanotube membrane can be extracted. The obtained results are in good agreement with the data reported by different techniques for similar lipid compositions. Hence the electric field can be utilized for measurement of mechanical parameters of tubular membrane, specifically, short and/or narrow tubules which are not readily accessible by conventional techniques.

1807-Pos Board B651

Surface Behaviour of Peptoid Mimics of Pulmonary Surfactant Protein SP-C: Captive Bubble Surfactometry

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Pulmonary surfactant lipopeptide SP-C modulates the surface properties of interfacial films required to stabilize the respiratory interface along breathing dynamics. Several attempts have been made to produce entirely synthetic analogs of SP-C suitable to develop potentially useful therapeutic preparations.

In this study we have tested the potential of five different poly-*N*-substituted glycines, or peptoids, designed to mimic (roughly) the primary and secondary structure and hydrophobicity of SP-C, to produce acceptable surfactant-like behaviour once incorporated into lipid/peptoid suspensions and assessed in a captive bubble surfactometer (CBS). The surface activities of different peptoids were compared in two model lipid mixtures: DPPC/POPG/Palmitic acid (68/22/9), which resembles the lipid composition of several clinical surfactants currently in use, and DPPC/POPC/POPG/Chol (50/25/15/10), which mimics the balance of saturated/unsaturated and zwitterionic/anionic phospholipids and the cholesterol content of natural surfactant as purified from bronchoalveolar lavage. We have assessed the ability of the different lipid/peptoid suspensions to i) rapidly adsorb at the bubble air-liquid interface, ii) stably produce very low surface tensions upon relatively slow repetitive quasi-static compressions and iii) maintain the lowest surface tensions with minimal compression and hysteresis under rapid physiological-like compression-expansion dynamics. Significant differences were found between different peptoids differing in their backbone structure and hydrophobicity, with some of the peptoids mimicking efficiently the effect of native SP-C, usually at larger proportions of peptoids than required for the natural protein.

1808-Pos Board B652

Looking at Lipid Domains in Stratum Corneum Lipid Models using Vibrational Microspectroscopy

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The impermeability of the skin is intimately related to the structure of the stratum corneum (SC), the top layer of the epidermis. The large fraction of SC lipids existing in a solid/crystalline form is believed to be a key factor in the low permeability of the skin barrier. We have characterized, using Raman and infrared microspectroscopies, the mixing properties of model mixtures that included ceramide, free fatty acids, and sterol, the 3 main lipid components of SC. We show that, in ternary mixtures with palmitic acid and cholesterol, the transformation of sphingomyeline, a precursor of ceramide, into ceramide leads to an increase of the heterogeneity of the spatial lipid distribution, in parallel with an increase of the chain order. Therefore the enzymatic conversion of sphingomyeline in ceramide leads to the transformation of a homogeneous and relatively disordered matrix into a heterogeneous matrix containing crystalline domains. This heterogeneity in lipid composition was observed from the microscopic local variations of the relative areas of the C-H stretching and the C-D stretching bands, the fatty acids being deuterated in our model mixtures. The thermal evolution of the mixing properties of the ceramide/palmitic acid/cholesterol mixtures indicated that an increase of temperature (above 50 °C) leads to the disordering of the fatty acid and, to a lesser extent, of ceramide. In parallel to this melting, a mixing of the lipid species is observed as the areas enriched in palmitic acid were also enriched in cholesterol. These results suggest the formation of a liquid ordered phase mainly composed of palmitic acid and cholesterol; this phase may ensure the cohesion between the solid domains. The recording of spectra from several microscopic voxels provides a unique description of the phase composition of these model mixtures.

1809-Pos Board B653

Comparative Studies On Bovine And Rat Pulmonary Surfactants Using AFM

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Recent studies employing a variety of microscopic techniques have revealed that phospholipid (PL) phase separations and transitions may play important roles in determining the biophysical properties of pulmonary surfactant. Most of these microscopic studies used model systems which were composed of simple mixtures of PL with or without hydrophobic surfactant proteins and cholesterol. The present work compared modified natural (lipid extract) bovine and rat surfactants using atomic force microscopy (AFM). AFM revealed PL phase separation upon compression of both surfactant monolayers, and a monolayer-to-multilayer transition at surface pressure 40-50 mN/m. Similar to bovine surfactant, the tilted-condensed (TC) phase in rat surfactant consisted of domains both on micrometer and nanometer scales. Upon film compression, the microdomains were dissociated into nanodomains, thus forming a more homogeneous two-phase mixture. Differences between rat and bovine surfactants were: (1) more TC domains were formed at lower surface pressures in rat than in bovine surfactant; and (2) an interesting domain-in-domain structure was exclusively observed in rat surfactant. These structural differences were attributed to the higher cholesterol content of rat surfactant (~ 10 vs ~2.5 wt%). To further investigate the effects of cholesterol on the structure of surfactant films, we have studied cholesterol-depleted bovine surfactant (~ 0%) prepared by repetitive acetone extraction. Removal of cholesterol from bovine surfactant induced significant variations in film structure. More importantly, the film structure can be effectively restored by recombining cholesterol with the cholesterol-depleted bovine surfactant. Recombinant bovine surfactant with 10% cholesterol showed domain-in-domain structures similar to those found with rat surfactant. These interspecies studies of the micro- and nano- structures of natural pulmonary surfactants add insight into the biophysical interpretation of phospholipid phase transition and separation, in particular the role of cholesterol.

1810-Pos Board B654

Differences in Lateral Membrane Organization in Fibroblasts Expressing Low and High Levels of the Influenza Viral Protein Hemagglutinin

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Lateral organization in cell membranes is crucial for biological processes such as endocytosis, signaling, protein transport, membrane trafficking and viral infection. Hemagglutinin (HA) is an influenza viral envelope transmembrane protein which has been shown to be associated with the liquid ordered (l_o) phase. Fibroblasts that constitutively express HA, referred to as HAb2 cells, were used to characterize the lateral organization of HA. Since HAb2 cells have cell-to-cell variability in the membrane density of HA, cells with low and high expression levels of HA containing more consistent densities of HA were also used. Fluorescence correlation spectroscopy (FCS), confocal microscopy and fluorescence photoactivation localization microscopy (FPALM) were used to characterize membrane organization after labeling cells with fluorescent probes and/or transfecting with either EGFP-HA or Dendra2-HA. Preliminary FCS results show that the diffusion of the liquid-disordered (l_d) phase probe Lissamine Rhodamine DOPE is similar in cells with high and low HA expression levels i.e. the amount of HA present does not influence the diffusion time. Confocal microscopy was used to study the effect of HA expression level on the extent of phase separation observed after blebbing was induced by DMSO treatment. FPALM was used to obtain details about membrane organization at the nanometer length scale.

1811-Pos Board B655

Determination Of The Lipid Membrane Composition Of J774 Macrophages Cells Surexpressing Mrp Protein (resistant To Ciprofloxacin)

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Ciprofloxacin (CIP) is a fluoroquinolone antibiotic with an activity towards both extracellular and intracellular bacteria (Seral *et al.*, 2005). Diffusion and efflux processes modulate accumulation of this drug within eukaryotic cells. When J774 macrophages were grown in presence of ciprofloxacin, the antibiotic is subject to constitutive efflux through the activity of an MRP-related transporter (Michot *et al.*, 2004).

In view of the critical role of lipids for both drug uptake and activity of MRP proteins (Hinrichs et al, 2004), together with the ability of fluoroquinolones to interact with lipids (Bensikaddour *et al.*, 2008 (a,b)), we investigated the composition of lipids in resistant and sensitive J 774 macrophages to ciprofloxacin. Firstly, we characterized by thin layer chromatography the phospholipids composition of J774 macrophages cells sensitive (WT) and resistant to ciprofloxacin (CIP). Results showed that sphingomyelin (SM) decreased 2 times whereas phosphatidylinositol increased 1.5 fold in resistant cells. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and cholesterol didn't show any significant change. Secondly, we studied membrane fluidity of liposomes