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Condensing And Fluidizing Effects Of Structurally Related Gangliosides On Phospholipid Films

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In model membrane mixtures that mimic lipid raft compositions, the more ordered domains are enriched in the ganglioside, G_{M1}, which contains four neutral sugars and a negatively charged sialic acid. To understand the organization and partitioning of G_{M1} in cell membranes, the outer leaflet of the cell membrane was modeled using Langmuir monolayers of DPPC and varying concentrations of G_{M1}. At low biologically relevant concentrations, G_{M1} condenses the DPPC monolayer while at higher concentrations, it acts to fluidize, with a switch-over point between the two behaviors at a ratio of 3:1 DPPC:G_{M1}. To examine phase morphology and organization of the components, the monolayers were transferred onto solid substrates and imaged with atomic force microscopy. At concentrations below the switch-over point, G_{M1} is located in nanoscale clusters within the condensed DPPC domains. The total surface area of these nanosize domains is larger than that attributable to G_{M1} molecules alone, suggesting the regions are due to G_{M1} and DPPC packing preferentially in condensed geometric complexes.

To pinpoint the structural region of G_{M1} giving rise to the condensation effect, parallel experiments were run with ceramide and PEGylated lipids. Our results indicate that the bulky, rigid sugar ganglioside headgroup is necessary for the significant phase behavior effects on the surrounding lipid molecules. Ganglioside headgroup geometry and charge were further explored with binary mixtures of asialo-, disialo- and trisialo-gangliosides (containing zero, two, and three sialic acids) with DPPC. In all cases, a similar condensing and fluidizing effect that varies with ganglioside concentration is seen, suggesting the negatively charged sialic acid residue is not critical for the close-packing phenomenon. Variations in mole ratio of critical packing between different ganglioside molecules can be explained by global effects of headgroup geometry, charge, and resultant molecular dipole moments.

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Nanoscopic Rearrangement Of Outer And Inner Leaflet Membrane Proteins Due To Ige Receptor Cross-linking

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Antigen-mediated cross-linking of immunoglobulin E (IgE) bound to its high affinity receptor FcεRI on mast cells initiates a transmembrane signaling cascade that results in cell activation and exocytotic release of chemical mediators involved in allergic response. Plasma membrane lipids and proteins redistribute as part of this transmembrane signaling process. To understand the functional role of these redistributions, resolution of their size, composition and structure on the nanometer scale is required. We utilize high resolution scanning electron microscopy (SEM) to directly visualize sub-micron membrane domains in intact cell membranes. In our experiments, the distribution of gold-labeled proteins and lipids is analyzed at the surface of intact fixed cells using backscattered electron detection. In parallel, we also observe membrane topography using secondary electron detection. We use a pair-correlation function analysis to quantify protein distributions and parameterized domain size. We have mapped the distribution of a variety of proteins, both related and non-related to the IgE signaling pathway. Using this experimental and quantitative method, we observe dramatic changes in the nano-scale membrane distribution of IgE due to stimulation with multivalent ligands. In resting cells, IgE receptors are clustered into small domains of less than 30nm. Following receptor cross-linking, receptors are rapidly redistributed into large domains which are correlated at long length-scales. Additionally, we observe cross-linking dependent rearrangement of several inner leaflet-associated proteins that are implicated in early signaling events. In contrast, outer leaflet GPI-linked proteins are not affected. These findings demonstrate selective nanoscopic reorganization during the initiation of receptor signal transduction.

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Cholesterol Content And Domain Formation As Regulators Of PLA2-IIA Activity In Anionic Membranes

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Phospholipase A_2 type IIA (PLA_2-IIA) hydrolyses the sn-2 position of glycerolipids to produce free fatty acids and lysolipids. The enzyme presents a strong affinity towards membranes enriched in anionic lipids, and lipid-packing is known to influence the ability of PLA_2-IIA to extract lipids from the membrane. This leads to an interrelation between membrane structure, and enzyme activity. In this study we evaluate the activity of PLA_2-IIA on unilamellar vesicles composed of either 1- palmitoyl-2-oleoyl phosphatidylglycerol (POPG) or dimyristoyl - phosphatidylglycerol (DMPG), in combination with cholesterol (Chol) and sphyngomyelin (SM). We expose the vesicles to PLA_2-IIA and monitor the activity at 37°C, where both POPG and DMPG are in the liquid-disordered phase in their pure form. For DMPG/Chol or POPG/Chol the results show that adding cholesterol alone inhibits PLA_2-IIA activity. However, this effect is more accentuated in DMPG vesicles compared to POPG vesicles. We attribute this difference to a closer interaction of cholesterol with the saturated acyl chains of DMPG, leading to tighter lipid packing and a reduced hydrolysis rate. In the second part of the study, for POPG and DMPG samples in which we included cholesterol and sphyngomyelin in equal molarities, we detect high hydrolytic activity in a wider range of compositions for a given Chol/PG ratio compared to samples without SM. We propose that a strong affinity between SM and cholesterol, related to liquid-ordered domain formation, leads to a depletion of cholesterol from the PG-rich regions, maintaining a high PLA_2-IIA hydrolysis rate. In the third part of our study, we focus on changes in rigidity of the membranes after exposure to PLA_2-IIA based on Laurdan General Polarization measurements. We find a general increase in rigidity following the hydrolytic burst in POPG binary and ternary systems.

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Comparison of Insertion and Folding of Chaperone-bound Outer Membrane Protein A (OmpA) of E. coli into Phospholipid Bilayers of Various Composition

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OmpA spontaneously inserts and folds into lipid bilayers from a urea-unfolded state upon urea-dilution. Previous work demonstrated that urea can be replaced effectively by the periplasmic chaperone Skp when lipopolysaccharide (LPS), a component of the outer membrane, is present [1]. Skp was shown to bind outer membrane proteins with nanomolar affinity and to prevent their aggregation [2].

Here we investigated folding of Skp-bound OmpA into lipid bilayers of different headgroup composition and chain-length, both in absence and presence of LPS. For urea-denatured OmpA and in absence of Skp and LPS, kinetics of folding into bilayers containing the negatively charged phosphatidylglycerol showed a lag-phase of up to 30 min for dilauroylphospholipid bilayers (LUVs, 100 nm diameter) prior to folding. Skp inhibited folding and prolonged the lag-phase at basic pH when LPS was absent. In presence of LPS, no lag-phase was observed and folding rates increased dramatically.

When similar experiments were performed with bilayers composed of the corresponding dioleolylphospholipids (SUVs), a lag-phase was not observed, but Skp inhibited very strongly. LPS again stimulated OmpA insertion and folding, albeit not as much as observed for dilaurylphospholipid bilayers.

Skp inhibited folding also in experiments with neutral phosphatidylcholine bilayers, irrespective of lipid chain-length. Here, LPS could also facilitate folding of Skp-bound OmpA, but the effect was less pronounced for dioleoylphosphatidylcholine bilayers. The data suggest, LPS-assisted folding of Skp-bound OmpA depends on both, the surface charge of the membrane and on the lipid-chain composition.

References:
1. Bulieris, P. V., et al. (2003). J Biol Chem 278, 9092-9.
2. Qu, J. et al. (2007). J Mol Biol 374, 91-105.

2319-Pos Board B289

Binding Sites of Outer Membrane Protein A (OmpA) in the Complex with the Periplasmic Chaperone Skp from E. Coli. A site-directed fluorescence study

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The periplasmic chaperone Skp facilitates folding and insertion of membrane proteins into the outer membrane of Gram-negative bacteria [1,2]. We have studied the binding sites of OmpA in complex with Skp or with Skp and LPS [3] in aqueous solution by site-directed mutagenesis and fluorescence spectroscopy. Single tryptophan mutants of OmpA were prepared and isolated in unfolded form in 8 M urea solution. In thirteen mutants, the single tryptophan was introduced at different positions, namely in 5 of the 8 β-strands, in the 4 outer loops, and in the 3 turns of the 170 residue transmembrane domain and in addition in the 155 residue periplasmic domain. All mutants folded upon

dilution of the denaturant urea into lipid bilayers. In solution, fluorescence of the tryptophan introduced into the C-terminal periplasmic domain was strongly quenched and not significantly affected by Skp binding. Upon addition of a 4-fold molar excess of Skp, fluorescence of mutants with a Trp in the N-terminal domain was strongly increased and spectra were blue-shifted in comparison to aqueous forms in absence of Skp. Skp obviously bound to the entire N-terminal (β-barrel) domain. Fluorescence spectra of the single tryptophan located in strands, loops, and turns were differently affected by Skp binding and spectroscopic parameters changed in a periodic fashion, reflecting the locations of the tryptophans. Loop 1 and loop 3 of OmpA were found to preferably interact with LPS. Fluorescence of Trp in strands or turns of OmpA was not affected by LPS binding.

[1] Bulieris, P. V., et al. (2003). J Biol Chem 278, 9092-9.
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2320-Pos Board B290

Super-resolution Imaging Of Hemagglutinin Clusters In Cell Membranes

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The clustering of the influenza protein hemagglutinin (HA) in the viral membrane is necessary for membrane fusion and entry of the virus into host cells. Because HA is also associated with lipid rafts, controversial membrane structures which are involved in a variety of normal cellular functions, the mechanism by which HA ''hijacks'' normal cell membrane rafts for its own purposes is of great interest. However, due to the limitations imposed by diffraction on spatial resolution in light microscopy, the properties and even the existence of rafts have remained elusive. Using fluorescence photoactivation localization microscopy (FPALM) it has been possible to obtain super-resolution images of the distribution of HA in living and fixed fibroblast cell membranes with resolution nearly an order of magnitude better than conventional fluorescence microscopy. This novel method yields time-resolved nanoscale dynamics, orientational information, and other single molecule properties for large numbers of ($> 10^4$) molecules in living and fixed cells. In combination with quantitative analysis, FPALM imaging of the dynamic distribution of HA provides a means to test several current models of membrane raft organization.

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Lipid Domains in Bacterial Membranes as a Predictor of Antimicrobial Potency

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- A wide range of chemical structures having antimicrobial activity have been studied in an effort to treat the increasing emergence of bacteria that are resistant to traditional antibiotics. These agents have varying degrees of toxicity against different bacterial species. We demonstrate, using members of the novel class of antimicrobial agents, the oligomers of acyl-lysine (OAKs), that one cause for the difference in species selectivity is the ability to induce the clustering of anionic lipids, resulting in their segregation into domains. We demonstrate by DSC and by MAS/NMR that a membrane-active OAK is capable of inducing lateral phase separation in mixtures of anionic and zwitterionic lipids. Such a phenomenon would occur only in bacterial membranes composed of both anionic and zwitterionic lipids and not with bacteria whose membrane lipids are largely anionic. As a consequence it can be predicted which bacterial species will be most affected by antimicrobial agents that function principally by this mechanism. We demonstrate that this criterion provides an explanation for the greater toxicity of certain OAKs against Gram negative bacteria, in spite of the presence of an outer membrane. This finding allows for the design of new antibiotics with selective toxicity against different groups of bacteria.

2322-Pos Board B292

Gene Silencing Activity of siRNA Embedded in a Bicontinuous Lipid Matrix

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Small interfering RNAs (siRNAs) are short (19-29bp) double stranded nucleic acids that efficiently mediate gene knockdown in mammalian cells by directing the degradation of complementary target mRNA sequences. This has implications in a vast number of fields involving therapeutics, gene identification and function.

The transport of exogenous nucleic acids into a host cell requires a suitable carrier; examples of which include synthetic and viral vectors. Synthetic cationic lipid (CL) assemblies can efficiently be used for transfection of DNA. Recently, we found that the same assemblies can be used to deliver siRNA, leading to highly specific gene silencing [1].

The ability of siRNA-lipid aggregates to proficiently silence genes is strongly correlated with the amount of CL in the complex. Specifically, the number of CL per siRNA must be sufficiently large to pack the nucleic acid while remaining below a limit that induces cell toxicity. In contrast to long DNA, siRNA fails to efficiently pack in a liquid crystalline fashion in the 2D lipid matrix. Hence, larger amounts of CL are required to pack siRNA. This potentially leads to undesirable cytotoxicity of the lipid carrier.

Our current work circumvents this problem by increasing the dimensionality of the lipid matrix hosting the nucleic acid. We successfully stabilized a 3D bicontinuous lipid phase containing siRNA. This lipid carrier efficiently delivers siRNA even at low amounts of CL. The resulting complexes yields highly specific gene knockdown with a significant reduction in cytotoxicity.

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[1] Bouxsein et al. Biochemistry (2007) 46, 4785.

2323-Pos Board B293

Effect of Nucleic Acid Length and Chemistry on Structure-Function Properties of Cationic Lipid-Nucleic Acid Complexes

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The success of gene therapeutics is tied to the efficiency of transfer of the nucleic acid (NA) into a target cell type with minimal side effects. Synthetic vectors for delivery, such as cationic lipids (CL), can satisfy these criteria but only though a fundamental understanding of the structure-function relationship between CL-NA. The delivery efficiency of CL-DNA complexes follows a universal relationship that depends on the membrane charge density of the lipid layers but is independent of the type of CL. Our work on CL-siRNA complexes reveals stark differences in delivery behavior: the efficiency is not only broadly lower when compared with equivalent composition CL-DNA but also depends on the valence of the CL with multivalent favored over monovalent [1]. X-ray structural work indicates that siRNA ordering in CL-siRNA is isotropic, contrasting the smectic ordering of DNA in CL-DNA. We hypothesize that this isotropic behavior prevents optimal packing of the siRNA and is responsible for lower efficiencies. This behavior is in general agreement with Onsager rigid rod criteria for liquid crystal order transitions that depend on the dimensional anisotropy of the molecules. Our work with analogues CL-short DNA (sDNA) contradicts these findings; ordered sDNA phases can be seen in the 2D environment between the lipid layers well below the Onsager limit. This is consistent with recent work on sDNA suspended in bulk water [2]. While the ordered phase transitions of sDNA depend on length they also depend on the chemistry of the NA ends. These considerations may be applied to the siRNA in an attempt to improve the CL-siRNA packing efficiency.

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2324-Pos Board B294

Self-repair Of Bacterial Cell Wall Against Multiple Puncturings By An AFM Tip

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We report, for the first time, that bacteria survive multiple stabbings by an atomic force microscopy (AFM) tip under physiological conditions. Experiments were conducted using *Salmonella* Typhimurium as a model. The fimbriae (pili) of *S.* Typhimurium and the corresponding antibody were used to immobilize *live* bacteria in well-defined patterns on a flat substrate. A carefully calibrated AFM was used to conduct the experiments in a growth medium: An AFM tip with known radius was used to apply pressure to a bacterium with a known force until the tip penetrated the bacterium cell wall and reached the other side of the bacterium. This experiment, which generated a characteristic puncture curve, was repeated more than 50 times at different locations on the same bacterium. A MatLab® code was written to analyze the puncture curves, which carry an abundance of information on such characteristics of the bacterium as the surface elasticity, the critical pressure needed to puncture the bacterial cell wall, the interaction of the AFM tip with the interior of the