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.

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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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[1] Bouxsein et al. Biochemistry (2007) 46, 4785.
[2] Nakata et al. Science (2007) 318, 5854.

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