(62%), but the remaining 38% of digestion occurred in the course of the subsequent intermittent encounters. The complex formation was maintained after the DNA break. This is suggestive of an intrinsic behaviour in which the DNA and protein molecules are continuously held together by switching their binding positions with short-range interactions. It also provides a clue to understanding an efficient and effective reaction by the DNA-binding proteins in bacterial cells.

Membrane Physical Chemistry III

3546-Pos Board B274

Rapid Determination of Geometry and Elastic Constants of Lipid Nanotubes

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Membrane nanotubes (NT) are cylinders made of a lipid bilayer. They are widely used to study the effects of curvature on the mechanics of lipid bilayers and proteo-lipid interactions. Fast and accurate determination of the size (length and radius) and the elastic parameters (membrane tension and bending rigidity) of the tube is imperative to quantify its interaction with proteins. Here we describe how measurements of electrical conductivity of the NT interior under different voltage protocols allows for simultaneous real time measurements of its geometry and mechanical properties. We present the correlative analysis of conductance and fluorescence microscopy measurements performed on a single microns-long nanotube. For submicron nanotubes we discuss the precision of the method (which approaches 0.5 nm for the measurements of the NT radius) and demonstrate that bending moduli obtained here for the nanoconfined NTs of different lipid compositions correspond to those obtained from the bulk measurements.

3547-Pos Board B275

Interaction of Digitonin and Cholate with Complex Membranes

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The non-ionic detergent digitonin, extracted from purple foxglove, is widely used in the solubilization and reconstitution of membrane proteins. Its physical-chemical properties are not well characterized, however, and literature on its aggregation behavior is limited. In contrast, the bile salt sodium cholate which also is commonly used in the solubilization of membrane proteins, has been studied extensively. In order to understand the role of digitonin-lipid interactions in the reconstitution of G protein-coupled receptors, we have studied the interactions between a particular digitonin-cholate mixture and a mixed membrane composed of phosphatidylcholine, phosphatidylserine and cholesterol. Isothermal titration calorimetry was used, along with time-resolved fluorescence leakage assays and light scattering, to study the self-assembly of the mixed-surfactant system and its interactions with lipid membranes. The mechanism by which the digitonin-cholate surfactant mixture aids in the reconstitution of membrane proteins will be discussed. The insights gained from this work will facilitate the selection of detergents in future studies on the solubilization of membrane proteins.

3548-Pos Board B276

Membrane Leakage and Antimicrobial Action of Polymers and Surfactants

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Surfactants and nylon-type polymers have been found to induce leakage of lipid membranes as detected by dye efflux from liposomes. They also show inhibitory or cytotoxic activity against living cells. We aim at better understanding the quantitative correlation between these two activities. We hypothesize that this correlation depends crucially on the mechanism of membrane leakage. The graded mechanism involves partial efflux of entrapped dye from all vesicles, suggesting that it is based on frequent yet very small and short-lived defects distributed over all liposomes. Partial efflux by the "all-or-none" mechanism means that some liposomes leaked out all entrapped dye whereas others remained fully intact. This scenario implies the existence of distinct pores that develop in some of the liposomes and remain there during the incubation time of the experiment. The experiments are done using the fluorescence lifetime-based leakage assay.

3549-Pos Board B277

Influence of Cholesterol Microstructures on Fluctuation Spikes in Nystatin Channel Currents in Phospholipid/Cholesterol Bilayers

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Nystatin (NYS) is an antifungal agent that preferentially forms ion channels in membranes containing ergosterol (ERG) or cholesterol (CHOL). In phospholipid bilayers ERG or CHOL segregate into ordered (L_o) and disordered (L_d) domains. We prepared POPC bilayers containing CHOL mol fraction $0.18 \leq \chi_{ERG} \leq 0.50$ separating two chambers containing KCl solutions. The concentration gradient between chambers was 435/150 mM KCl. We allowed the CHOL/POPC bilayer to settle 15 min then added 19 or 38 microM NYS to the reference (Cis) chamber, imposed 50 mV across the bilayer, and stirred the Cis chamber at 4 Hz. The NYS molecules adhered to the bilayer forming channels on the boundaries of the L_o domains. [1] The resultant bilayer current exhibited prominent spikes of very short duration. A plot of the frequency of these spikes against χ_{ERG} revealed prominent spikes at $\chi_{ERG} = 0.19$, 0.25 and 0.40. These correspond well to the dips in dehydroergosterol fluorescence observed by Chong [2], which he understood in terms of a superlattice. We conclude that fluctuations in NYS channels on the perimeter of the L_o CHOL domains depend strongly on L_o domain structure.

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2. Chong, P.L-G. 1994. Evidence for regular distribution of sterols in liquid crystalline phosphatidylcholine bilayers, Proc. Nati. Acad. Sci. USA 91:10069-10073.

3550-Pos Board B278

Optimizing Drug Release: Bilayer to Inverted Hexagonal Phase Transition of Cationic XTC2 and Anionic DSPS Lipid System is Influenced by pH, Temperature, and Salt Concentration

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The effectiveness of a macromolecular drug delivered in lipid nanoparticles (LNP) depends upon the biophysical properties of the delivery vehicle. Recent research has shown that the design of the cationic lipid component of LNPs improves the intracellular delivery of therapeutic siRNA[1]. Even in these optimized LNPs, only 1% of the siRNA taken up by the cell via endocytosis is actually released into the cell cytosol. We proposed a mechanism of endosome disruption that relies on the formation of non-bilayer phases in the presence of anionic endosomal lipid and synthetic cationic lipids. A model system using prototypical anionic lipid 1,2-distearoyl(d70)-*sn*-glycero-3-[phospho-L-serine] (DSPS-d70) in 1:1 molar ratio to the cationic lipid DLin-KC2-DMA (XTC2) (pKa~6.7) was characterized by ^2H and ^{31}P NMR spectroscopy. Through spectral analysis, we determined that at physiological pH (~7.4) the XTC2/DSPS system exhibits a stable gel phase for temperatures below 45°C while an isotropic signal emerges at higher temperatures - no inverted hexagonal (H_{II}) phase is observed. At low pH (~4.75), the XTC2/DSPS system is principally in a bilayer gel phase at low temperatures with a non-bilayer H_{II} phase predominating at higher temperatures. The transition from gel to H_{II} phase is dependent on salt concentration and is most evident in the range of 15-25°C for 0.25M [Na^+], 20-30°C for 0.5M [Na^+] and 35-45°C for 1M [Na^+]. Through depaking the spectra, order parameter profiles S_{CD} have been obtained and compared for DSPS-d70 chains in bilayer and H_{II} phases. These will be useful for computational simulation and eventually to design *in vivo* animal model experiments.

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3551-Pos Board B279

Modelling of the Interaction between Cationic Lipid Dlin-Kc2-Dma (XTC2) and Anionic Lipid Distearoylphosphatidylserine (DSPS)

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Short pieces of double stranded small interfering RNA (siRNA) could be used as a potential drug to cure cancer by binding to cancer gene's messenger RNA. However, the siRNA is not stable in the blood stream, and tends not to penetrate target cell membrane. So, it must be encapsulated in a lipid nanoparticle. LNPs have been shown to be strong candidates for drug delivery [1]. Lipid

nanoparticles (LNPs) are a new tool for drug delivery systems. They are responsible for transferring short pieces of double stranded small interfering RNA (siRNA) into the cell. However, the underlying mechanism of action of lipid constituents of LNPs is not clearly known. Specifically, characteristics such as encapsulation efficiency, and the stability of LNPs are not predictable. Cationic lipids play essential roles in encapsulating negatively charged siRNA and also destabilizing the endosomal membrane. We have studied the bilayer and inverted hexagonal phase (HII) phase stability as it could potentially show the destabilizing effect of LNPs on the endosomal membrane for DLin-KC2-DMA (XTC2) at different temperatures. It has shown good destabilizing effects for bilayer structures. We also studied the interaction of the distearoylphosphatidylserine (DSPS) with XTC2 based on comparison with deuterium order parameters obtained from NMR experiments since DSPS has been used to optimize the structure of XTC2 [1]. pH, water content, temperature, and salt concentration effects on the bilayer HII phase stability is also studied.

[1] S. de Youg, G. C. Chikh, L. L. Sekirov, S. Raney, S. C. Semple, S. K. Klimuk, Z. N. Yuan, M. Hope, P. R. Cullis, Y. K. Tam, Cancer Immunology and Immunotherapy, 56, 1251-1264, 2007.

3552-Pos Board B280

Interaction of Mefloquine Hydrochloride with Cell Membranes Models Studied with Tensiometry and Vibrational Spectroscopy

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Mefloquine hydrochloride is a drug used in the treatment of malaria, with a probable effect on cell membrane surfaces. However, the mechanism of action when they interact with lipid surfaces is not sufficiently known so far. For this reason, it is important the understanding at the molecular level of drug-membrane interactions, and using models for biointerfaces membranes is a suitable strategy for this purpose. In this study, we employed Langmuir monolayers of lipids as biointerface models, with the drug incorporated in monolayers of zwitterionic lipids, namely DPPC (dipalmitoyl phosphatidyl choline), negative lipids, namely DPPS (dipalmitoyl phosphatidyl serine), and cholesterol. Combining data on Surface Pressure-Area Isotherms with Polarization Modulation Infrared Reflection-Absorption Spectroscopy (PM-IRRAS), the effect of the mefloquine hydrochloride on lipid monolayers was compared by taking into account the chemical and molecular structure of the lipids and the drug. The adsorption of the drug at the monolayers decreases the order of the lipid film, replaces water molecules from the interface, in mechanisms that involve both polar head groups and alkyl tails from the lipids. A model is then proposed in which mefloquine hydrochloride interacts with lipids at the air-water in such a way that the interactions are maximized owing to geometrical adaptations on behalf of the contact between lipid polar heads and polar drug groups.

3553-Pos Board B281

Effect of Phospholipid Charges and Spacing on Kinetics of Laurdan and Patman Equilibration with Phospholipid Membranes

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The steady-state fluorescence of Laurdan and its cationic derivative, Patman, is commonly used to assess environmental polarity of phospholipid bilayers. Recent studies have demonstrated that analysis of the pre-steady-state kinetics of probe fluorescence provides additional information about heterogeneity and biophysical properties of probe configurations within the membrane. Since this configurational heterogeneity is more prominent with Patman than Laurdan, studies were conducted to identify the roles of membrane charge on the equilibration process. Emission intensity of Patman and Laurdan was monitored at 435 and 500 nm at various temperatures during equilibration with unilamellar vesicles or micelles with different charge densities. To isolate the effects of positive charge, comparisons were made between phosphatidylcholine and phosphatidylglycerol. The role of the negatively-charged phosphate was investigated by varying the sample pH. In general, equilibration of Patman followed two kinetic processes whereas Laurdan equilibration was faster and displayed first-order kinetics. Arrhenius plots of the initial rate of Laurdan equilibration were linear with negative slopes under all conditions. However, with Patman, Arrhenius profiles were more complex. Arrhenius plots with phosphatidylglycerol bilayers were flat, discontinuous at the main phase transition, and faster in the fluid phase. With phosphatidylcholine bilayers, Arrhenius slopes were steeply negative at high temperatures and flat at lower temperatures. The discontinuity occurred above the melting temperature and disappeared at low pH. Equilibration of Patman with micelles was generally faster than with vesicles and was instantaneous with phosphatidylglycerol at low pH. These data suggest at least three conclusions. First, the main energy barrier to Patman equilibration with phosphatidylcholine is the positively-charged choline. Second, the negatively-charged phosphate causes Arrhenius slope discontinuities. Finally, in the absence of charge, equilibration is limited by the density of lipid packing.

3554-Pos Board B282

Interaction of Semiflexible Filamentous Virus Particles with Freestanding Lipid Membranes

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Understanding of the mechanisms of interaction of macromolecules and colloidal particles with lipid membranes is far from complete, and the questions related to role of local perturbation of the membrane properties in these interactions are still largely unsolved. Previously, we have found [1] that interaction of DNA molecules with strongly charged freestanding cationic lipid bilayers [2] leads to an unexpected phenomenon of membrane-mediated coil-globule transition of membrane-absorbed DNA macromolecules. To elucidate the effect of the persistence length in these phenomena, we study the behavior of much stiffer semiflexible *fd* virus particles (persistence length ~2.2 μm) electrostatically adsorbed on freestanding cationic lipid membranes. At low membrane charge densities, membrane-adsorbed *fd* virus particles behave as semiflexible filaments in 2D. On the other hand, we find that membrane-driven interactions at higher membrane charge densities are strong enough to induce the membrane-mediated coil-globule transition of the relatively stiff *fd* virus particles, which agrees with the recent theoretical predictions [3]. Further, for *fd* virus particles adsorbed at higher surface densities on weakly charged membranes we observe a new unexpected phenomenon of membrane-driven self-organization of the filamentous virus particles into long linear chain aggregates.

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[2] C. Herold, G. Chwastek, P. Schwille, and E. P. Petrov, *Langmuir***28** (2012) 5518.
[3] A. G. Cherstvy and E. P. Petrov (2013) submitted.

3555-Pos Board B283

Diffusion and Freezing Transition of Rod-Like DNA Origami on Freestanding Lipid Membranes

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During the last decade, DNA origami has become a powerful tool in research at the nanoscale. The relative ease of constructing functionalized DNA origami structures of a defined shape allows for their applications in membrane biophysics. Recently, we have constructed stiff rod-like DNA origami structures consisting of six DNA helixes, which were functionalized with hydrophobic membrane-binding anchors and fluorescently labeled at defined positions [1]. Selective fluorescent labeling allowed us to determine the translational and rotational diffusion coefficients of the DNA origami rods on lipid membranes by fluorescence correlation spectroscopy, which were found to be in a good agreement with the hydrodynamics-based theory of membrane diffusion. Further, we studied the effect of the surface density of membrane-bound origami structures on their Brownian motion and found a strong decrease of the translational and rotational diffusion coefficients of membrane-bound nanorods with an increase in their surface density. We compare our experimental findings with results of Monte Carlo simulations of Brownian hard needles in 2D.

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3556-Pos Board B284

Structure and Dynamics of Lens Lipid Membranes Derived from a Single Porcine Donor: High Field EPR Study

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To obtain correct EPR line shapes, spin-lattice relaxation times, and bimolecular collision rates between spin labels and oxygen, samples must be thoroughly deoxygenated or equilibrated with a controlled oxygen partial pressure. These measurements are conveniently carried out using a gas permeable plastic sample tube of small diameter that fits in a loop-gap resonator. Flow of gas over the tube allows easy deoxygenation or controlled oxygenation of the sample. In the initial design of the W-band (94 GHz) loop-gap resonator, samples were equilibrated with gas at room temperature outside the resonator, transferred to a quartz capillary, and positioned in the resonator. In a recently