topology. Thus we use the information derived from the database clustering to characterize structure elements as potential RNA building blocks. Together with techniques such as molecular dynamics simulations on selected structural motifs, we use this structure variability information to design RNA nano-scale structures. We present examples of the RNA nano-scale modeling process with emphasis on the characterization of the structural motifs used as building units as well as that of the entire structure.

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305-Pos How Large is a Large RNA?

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Board B138

Single-stranded (ss) RNA molecules are involved in virtually every part of the cell and in a wide variety of roles, e.g., as messenger RNA, transfer RNA, structural RNA, and enzymatic RNA. Furthermore, a large majority of viral genomes are ssRNA molecules, often as long as 10,000 nucleotides (nt) in length. In this work we address the question: what are the 3D sizes of large ssRNA molecules and how do they depend on nucleotide length and sequence? To determine their physical dimensions, we measure the radii of gyration (R_g) and hydrodynamic radii (R_h) of several natural and engineered RNAs whose lengths range from a few hundred to several thousand nucleotides.

We find that for a fixed nucleotide length the 3D sizes of RNAs with different base sequences can vary enormously. For example RNAs of length ~2000nt with roughly the same base composition can vary in R_g and R_h by over 30%. Moreover, by using low ionic-strength buffers to minimize tertiary interactions within the RNA molecule, we demonstrate that the size discrepancies need not arise from specific long range contacts, but instead are a generic property of the secondary structure, which in turn is determined by the nt sequence. Some viral RNAs that self-assemble into spherical protein capsids are presented as highly evolved cases where the primary sequence codes for a relatively compact size and shape. For example, the R_g of a 3000nt viral RNA from CCMV is of the order of that for a 1000nt non-viral sequence.

R_h's are obtained from fluorescence correlation spectroscopy (FCS) measurements and R_g's from small-angle x-ray scattering (SAXS). Pair distribution functions and Monte-Carlo reconstructions derived from SAXS studies are used to illustrate the intrinsic anisotropy of selected molecules and their ramifications for biological processes such as viral capsid assembly.

306-Pos Molecular Ångström Optics: A dynamical view of biomolecular structure

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Board B139

Using a confocal fluorescence microscope the newly developed multiparameter fluorescence detection (MFD) enables us to simultaneously collect all fluorescence information such as intensity, lifetime, anisotropy in several spectral ranges) from picoseconds to seconds. MFD is applied to perform single-molecule fluorescence-resonance-energy-transfer (FRET) studies on nucleic acids labeled with a fluorescent donor and acceptor dye. Thus, it is possible to circumvent the classical pitfalls of the FRET method in ensemble measurements. These novel FRET-based detection and analysis methodologies allowed us to resolve structural subpopulations with sub-nanometer resolution. Furthermore, direct access to the time trajectories of the different fluorescence parameters is obtained revealing the dynamics of the system. Finally, the construction of more-dimensional frequency histograms of the fluorescence parameters found in the trajectories on the single molecule level and selective analysis of these species (e.g. selective correlation analysis) give detailed view on the molecular energy landscape and the associated molecular structures.

Moreover a probability distribution analysis (PDA) method for calculating a priori histograms of FRET signals is presented taking explicitly crosstalk, stochastic variations and background into account. Histograms for the shot noise limited FRET signal are obtained by finding the mean as the only parameter in a least squares fit. Error analysis suggests an ultimate level of precision in determining separations with FRET of 1% of the Förster radius. The PDA method unambiguously distinguishes between stochastic processes and broadening due to signal heterogeneity. In this way quantitative structural information on various bent and kinked DNA and RNA structures was obtained. Moreover the structural and dynamic properties of the folding intermediate of a Holliday junction could be characterized. Finally single-molecule fluorescence studies on nucleic acid binding proteins will be discussed showing that MFD has developed to a powerful tool for Molecular Ångström Optics.

Membrane Physical Chemistry - I

307-Pos PEO-PPO Block Copolymer Vectors Do Not Interact Directly with DNA but with Lipid Membranes

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Board B140

Small angle neutron (SANS) and light scattering was used to study the interaction between fragments of double stranded deoxyribonucleic acid (DNA) and a synthetic triblock [poly(ethylene oxide)-poly(propyleneoxide)-poly(ethylene oxide)] amphiphilic polymer, known as L64, a potential vector for gene therapy. The mechanism of action of this vector is yet unknown. The contrast variation method was used to separate the partial structure factors of the different components in mixtures of triblock and DNA. It has been found that the copolymer and DNA molecules exhibit repulsive interactions. Further, the interaction between the copolymer and a model lipid membrane was investigated in order to explain the action of the vector. Electrical measurements on black lipid membranes indicated that the main effect of L64 as a vector is to permeabilize the cell's membrane.

References

J. Gau-Racine, J. Lal, M. Zeghal, L. Auvray "PEO-PPO block copolymer vectors do not interact directly with DNA but with lipid membranes" Journal of Physical Chemistry B*111* (33): 9900–9907 AUG 23 2007.

308-Pos Role of Multivalent Cations in the Self-Assembly of Phospholipid-DNA Complexes

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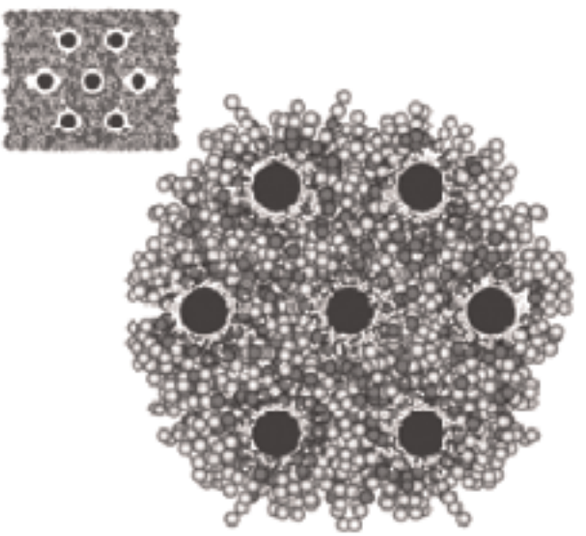
In view of efficient and non-toxic delivery of genes into cells, complexes made of phospholipids (non-cationic) and DNA are assembled through the mediation of multivalent cations. The association of lipids with DNA is explained through the charge reversal of lipid headgroups by specific adsorption of cations. The ion binding is quantified by the Gouy-Chapman-Stern theory which provides a good estimate for the minimal concentration of cations required to assemble complexes. Coarse-grained Monte Carlo calculations support small angle X-ray scattering experiments in the sense that lipids form inverted micelles around hexagonally-arranged DNA rods, with cations in between to maintain the cohesion (Figure). The complex cohesion, quantified by the minimized total free energy, becomes stronger as the cation valence increases and saturates beyond tetravalent cations. The presented methodology may help develop predictive models for biomolecular self-assembled systems, especially those used for gene delivery applications.

Figure. Self-assembly of a hexagonal lipid-DNA-ion complex by coarse-grained Monte Carlo simulations. The DNA rods, represented in dark, are fixed on a hexagonal lattice during the simulation. Zwitterionic lipids (gray spheres) and divalent cations (white spheres) are randomly distributed at the initial stage (inset).



309-Pos Modeling Calcium-Induced Adsorption of DNA onto Zwitterionic Lipid Membranes

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Board B142

Recent experiments suggest the ability of calcium to induce adsorption of DNA onto lipid membranes consisting of zwitterionic lipids such as phosphatidylcholine. We suggest a model that quantifies the adsorption strength and predicts the structural changes of the membrane at the DNA adsorption sites. Our model is based on Poisson-Boltzmann theory which we have modified so as to account for the dipolar character of the zwitterionic lipids. We find that calcium effectively screens the phosphate groups of the zwitterionic lipids, thus enabling the positively charged choline moiety to

interact with the DNA strands. Our results are consistent with experimental observations; they are based entirely on electrostatic considerations on the mean-field level without invoking the formation of complexes through the bridging of two zwitterionic lipids by one calcium ion.

310-Pos Biophysical Mechanisms of Protein Recruitment To Raft Domains Studied Using Planar Model Membranes: Recruitment by Native Binding Ligands versus GPI-anchored Proteins

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Board B143

Lipid rafts, which are liquid-ordered domains enriched in sphingolipids and cholesterol, are believed to play a significant role in several cell biological processes, largely due to their ability to segregate membrane proteins on the cell surface. The current study explores the poorly understood topic of biophysical mechanisms of protein recruitment to raft domains in a model raft system based on planar polymer-supported membranes. In particular, we report on the raft recruitment of membrane proteins by their native binding ligands and by GPI-anchored proteins. Three different types of proteins have been reconstituted and studied:

(i) lipid raft-associated [GPI-anchored proteins FcγRIII and urokinase plasminogen activator receptor (uPAR)];
(ii) non-raft-associated [transferrin receptor (TfR)]; and temporarily raft-associated (integrins $\alpha_V\beta_3$ and $\alpha_5\beta_1$).

The domain-specific quantification of reconstituted membrane proteins (labeled with dye-conjugated monoclonal antibodies) was achieved with high sensitivity using a combined setup of epifluorescence microscopy (Epi) and confocal fluorescence correlation spectroscopy (FCS).A ligand-induced raft recruitment process was observed for FcγRIII, uPAR, and $\alpha_V\beta_3$ (binding ligands/membrane proteins: IgA/FcγRIII, urokinase plasminogen activator (uPA)/uPAR, vitronectin/$\alpha_V\beta_3$). In contrast, TfR and $\alpha_5\beta_1$ show recruitment to liquid-disordered domains after binding to their ligands, transferrin and fibronectin. Remarkably, the current study also provides experimental evidence for the raft recruitment of the transmembrane protein $\alpha_V\beta_3$ by the GPI-anchored uPAR. To verify such an intriguing GPI-based recruitment process, additional evidence for the necessary $\alpha_V\beta_3$-uPAR complexation was obtained by tracking uPAR (labeled with dye-conjugated monoclonal antibodies) with and without $\alpha_V\beta_3$ using wide-field single molecule fluorescence microscopy.

311-Pos Light-induced Macroscopic Domains In Complex Model Biomembranes Containing Unsaturated Phospholipids

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Board B144

Macroscopic phase diagrams of 3-component lipid bilayer mixtures containing cholesterol reveal major differences among the different types of lipids. Fluorescence microscopy imaging is useful for these studies, but is subject to artifactual light-induced domain formation that appears with features similar to genuine $\{L\alpha + Lo\}$ and is being gradually recognized. Here we report that mixtures of cholesterol together with POPC and a high-melting temperature PC or SM show different phase behavior from similar mixtures containing DOPC or di-phytanoyl-PC instead of POPC. In particular, with POPC there is no region of macroscopic phase separation of $\{L\alpha + Lo\}$ domains. Light-induced macroscopic domains can form slowly enough (over several seconds) that changes in domain features with time can be recorded. In other cases, typically with high concentrations of fluorescent dyes (e.g. much greater than 0.1 mole %), the artifact is fully developed and stable even at the earliest observation. This artifact can be greatly reduced by decreasing illumination intensity and lowering dye concentration to ~ 0.03 mole %. The use of the free radical scavenger n-propyl gallate can reduce the artifact, but this molecule enters the bilayer and itself perturbs the phase behavior. We suggest that the light-induced domain separation artifact might arise from pre-existing lipid clusters that are induced to coalesce, and therefore indicates highly nonrandom mixing of the lipid components.

312-Pos Lateral Organization of Hemagglutinin in Biological Membranes Does Not Correlate with the Fluid Phase Raft Model

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Board B145

Lateral organization in cell membranes is crucial for biological processes such as endocytosis, signaling, protein transport, membrane trafficking and viral infection. The fluid phase raft model is among several models proposed to describe membrane heterogeneity. This model hypothesizes that the cell membrane exists in two fluid phases: a liquid ordered (l_o) raft phase, which is enriched in

saturated lipids and certain proteins, and a liquid-disordered (l_d) phase. Fluid-fluid phase coexistence is expected to yield domains with rounded (perimeter-minimized) boundaries. We show that the fluid phase raft model does not describe the lateral organization of the influenza protein hemagglutinin (HA) in fibroblasts at room temperature. Cell membranes were labeled with fluorescent probes and/or transfected with either EGFP-HA or PA-GFP-HA and imaged using fluorescence microscopy. The fluorescent probes, which were shown to preferentially partition into either the l_o or l_d phase in model membranes, did not show a labeling behavior consistent with two-phase coexistence in cell membranes. We observed at least three types of probe labeling regions; this alludes to the existence of more than two types of membrane environments. Using a recently developed high-resolution light microscopy technique called FPALM (fluorescence photoactivation localization microscopy), we also show that HA in live and fixed cells forms membrane clusters that span more than two orders of magnitude (~40 nm to >10 μm) in length scale with irregularly shaped boundaries. Results from immunogold electron microscopy corroborate these findings. Taken together, these results show that the fluid-fluid phase coexistence raft model does not describe the membrane distribution of HA.

313-Pos Simulating is believing: Visualization of rafts in model membranes

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Board B146

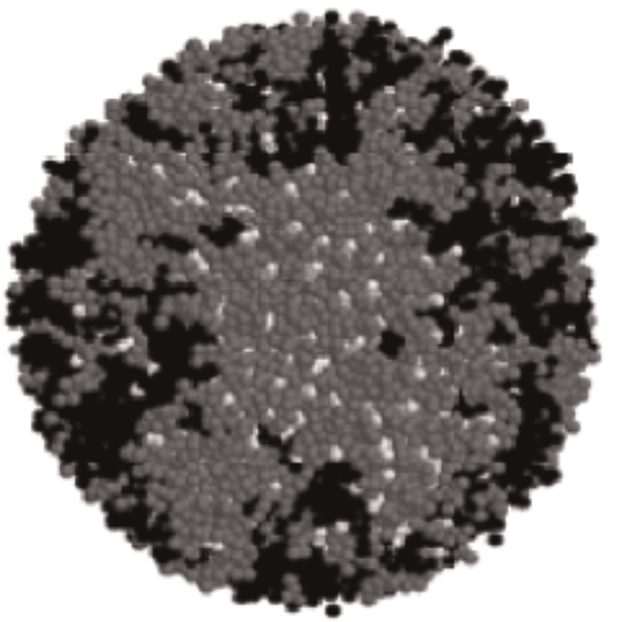
Whether or not domains formed by cholesterol and saturated lipids, so called rafts, play an important role in vivo remains a hotly debated topic. In model systems, however, phase separation in ternary lipid mixtures has been clearly visualized by an increasing number of experimental techniques (e.g. [1]).

To understand the molecular driving forces of raft formation, and to resolve the structural details of these nanodomains, computer modeling is essential. We use the coarse grained MARTINI force field [2] to simulate raft formation in small liposomes composed of saturated and polyunsaturated PC lipids together with cholesterol.

In agreement with experimental phase diagrams, at certain state points rafts appear spontaneously on a microsecond time scale.

We will present a detailed analysis of the formation and structure of these rafts at near-atomic resolution.

Figure: raft domain formed spontaneously in a small liposome. The overall composition is 12:7:8 (saturated-PC (gray) /polyunsaturated-PC (black)/cholesterol (white)). T=295K.



References

[1]. D. Brown. “Seeing is believing: Visualization of rafts in model membranes”, PNAS *98*:10517, 2001.

[2]. S.J. Marrink, H.J. Risselada, S. Yefimov, D.P. Tieleman, A.H. de Vries. “The MARTINI forcefield: coarse grained model for biomolecular simulations”, JPC-B, *111*:7812, 2007.

314-Pos GUV studies on Shape Morphology and Phase Perturbations

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Board B147

The well-know biconcave shape of the red-blood cell has been the subject of several computational investigations into its shape transformation from discocyte to stomatocyte to echinocyte. In Sheetz and Singer classic study (PNAS 71, 4457-4461 (1974)), perturbations to the exterior lipid leaflet of the membrane promotes the cup-shaped stomatocytes while additives to the interior have the opposite effect. ADE (area-difference elasticity) theory on vesicle shape points to leaflet area asymmetry, bending elasticity and volume as determining variables. We have uncovered parameters to promote area-difference between the lipid bilayer leaflets in vesicles and have mimicked the RBC discoid. Our analysis isolates the interface energetics of why the shape is favored and points to transient pores

enabling the vesicle to deflate and to assume a lower profile. Membrane fluidity and phase is key to the RBC transformations. We have begun to analyze additives, such as amphiphilic drugs. As we vary lipid composition and phase and as we intercalate additives, our RBC model system responds to these perturbations.

315-Pos Pressure-jump Time-resolved X-ray Diffraction Studies of Phase Transitions in Monoacylglycerols

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Board B148

Non-lamellar phases and phase transitions of membrane lipids are now believed to play important structural and dynamical roles in various fundamental cellular processes. In the past two decades the geometric pathways involved in such transformations (including lamellar-cubic and cubic-cubic transitions) have been extensively modeled. However, the systematic experimental data in pure lipid systems required for verification of such models is still lacking.

We have used pressure-jump time-resolved X-ray diffraction to investigate both the fluid lamellar - cubic (gyroid Ia3d) transition and the cubic-cubic (gyroid Ia3d - double diamond Pn3m) transition in hydrated monoacylglycerol model membrane systems.

We discuss the structural changes associated with these phase transitions which we have deduced. In addition, we have determined the transition kinetics, in both the forward and reverse directions, as a function of pressure-jump amplitude, temperature and water content. Trends in the transition rate with temperature and water content are shown to strongly reflect the shift in position of the phase boundary induced by a change in these thermodynamic parameters.

316-Pos Modulation Of The Phase Behavior Of Mixed Lipid Membranes By Adsorbed Proteins: A Thermodynamic Model

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Regulation of size and stability of membrane domains is a task to which membrane-associated proteins are likely to contribute. A generic scenario is the specific binding of proteins to one lipid species of a mixed membrane and the subsequent formation of protein-decorated domains. To gain insight into the underlying driving forces we suggest a thermodynamic model for the stability of a protein-decorated binary lipid layer. The model is based on a standard mean-field lattice-gas description for both the lipid mixture and the adsorbed protein layer. Besides accounting for lipid-lipid and lipid-protein interactions, it also includes direct protein-protein interactions. The result of our analysis is the characterization of the decrease in the membrane's critical point as a function of lipid-protein binding strength. For small and large binding strengths we provide analytical expression, numerical results cover the the intermediate range. Our results reiterate the crucial importance of the line tension, associated with protein-induced compositional gradients within the membrane. Beyond that, we demonstrate that direct protein-protein attractions can contribute substantially to the protein's ability to demix the membrane.

317-Pos Surface Rheology and Phase Transitions of Monolayers of Phospholipid/cholesterol Mixtures

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Board B150

The surface dilational viscosity and the dynamic surface elasticity of three binary phospholipid/cholesterol mixtures are determined with axisymmetric drop shape analysis (ADSA) on a harmonically oscillating pendent drop. Dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC) and dioleoylphosphatidylcholine (DOPC) are used to explore rheological properties and phase transitions of mixtures of these saturated and unsaturated phospholipids with cholesterol in various binary mixing ratios at room temperature, for DMPC at 20, 25 and 30°C. Growth rates of surface dilational viscosity and dynamic elasticity are parallel for all studied film pressures Π. Characteristic breaks and plateaus can be found with growing Π indicating phase transitions. For DPPC/cholesterol and DMPC/cholesterol mixtures phase diagrams with six regions separated by phase boundaries are found. These phase boundaries are in good agreement to transitions reported in the literature for static elasticity measurements on a Langmuir film balance and fluorescence microscopy. All phase boundaries are described by at least two independent authors and mostly by independent methods. Imaging methods reveal phase separations produced by the formation of condensed stoichiometric complexes leading to micron sized and mostly circular domains seen as two separated liquid phases in the so called α- and β-regions. The upper bound of the α-region is only detectable by fluorescence imaging, not by elasticity measurements. Phase transitions above the α-region at higher Π lead to a coexistence of a liquid and a solid phase and eventually a single solid phase (for DMPC only at 20°C). The upper bound of the β-region can be described both by fluorescence imaging and dynamic elasticity measurements. Above this phase boundary the existence of two separated liquid phases can be detected with charged fluorescent probes, not with uncharged. Dynamic elasticity shows a rheological behaviour above the β-region which is very similar to pure cholesterol.

318-Pos Tie-Line Determination for the Coexistence of Liquid Ordered and Liquid Disordered Phases

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Board B151

Saturated sphingomyelin (SM) lipids and cholesterol (Chol) are implicated in lipid rafts in cell plasma membranes. At room temperature and certain composition, membranes composed of unsaturated phosphatidylcholine (in our case Dioleoylphosphocholine or DOPC), SM and Chol, exhibit phase separation expressed in the formation of domains of liquid-disordered and liquid-ordered phases. Many recent studies focus on characterizing the coexistence regions in the phase diagram using giant vesicles as model membranes. When the latter are prepared from a three-component lipid mixture, DOPC/SM/Chol, the composition of the different vesicles in a batch can vary drastically depending on the individual history of the vesicles, which is *a priori* unknown. To deal with this problem we propose to use fusion of vesicles made of two components only (either DOPC and Chol, or SM and Chol) in order to obtain a vesicle of exactly known composition. The vesicles are differently labeled with fluorescent dyes and prepared separately. After mixing the two vesicle populations, the fusion is achieved by bringing together two vesicles of different composition and applying a strong electric pulse. Knowing the area and composition of the two fusing vesicles, we can calculate the exact composition of the final fused vesicle. We then follow the redistribution of the lipids between the two phases expressed in a change in the domain size in order to determine the location of the tie lines in the coexistence region of liquid ordered and liquid disordered phases. The vesicle domains are detected with confocal microscopy and their area is determined with home-developed software for image analysis.

319-Pos Effects of Hydrostatic Pressure on the Structure and Phase Behaviour of Natural Sphingomyelin Extracts

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Board B152

Sphingomyelin (SM) is an important phospholipid component of the plasma membrane, where it has been implicated in the formation of lipid rafts. In cholesterol-containing model membranes it tends to associate with phase-separated liquid-ordered domains in the bilayer. Despite its importance there is still relatively little data available on its lyotropic phase behaviour. This is partly due to SM often being obtained as natural extracts from a range of sources, each containing a different mixture of SM species. SM is based on a sphingosine backbone, which contributes one of the two chains present; the second chain is variable and differs in both carbon chain length and degree of unsaturation. Extracts from different sources have very different chain compositions, and this may result in different phase behaviour.

Using synchrotron x-ray diffraction and a high-pressure X-ray cell we have investigated and compared the phase behaviour of SM extracts from bovine brain, egg yolk and milk over a range of hydrostatic pressure. We observe significant differences in the structure of the gel phases, which can be linked to the difference in the chain composition of the extracts.

We have also initiated pressure-jump time-resolved X-ray experiments in order to investigate how the kinetics of the gel-fluid transition is affected by the nature of the gel phase immediately below the fluid lamellar phase.

320-Pos The Effect Of Cholesterol On Lipid Flip-flop And Desorption

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Board B153

Lipid trafficking is a fundamental process in cellular membranes for maintaining the appropriate composition of lipids, interacting with proteins, and participating in cellular signaling. In addition to lateral diffusion, lipids move between leaflets of same bilayer, a process called flip-flop, as well as between different bilayers, which involves desorption from the donor bilayer. Cholesterol is an important component of most eukaryotic cellular membranes, ranging from 0–40 mol% concentration between different membranes. Cholesterol is known to be important for regulating membrane fluidity, cellular signaling, and is speculated to be a key player in lipid rafts. The effect of cholesterol on lipid trafficking was studied using molecular dynamics (MD) simulations. Potentials of mean force (PMFs) were calculated for dipalmitoylphosphatidylcholine (DPPC) and cholesterol partitioning through DPPC bilayers containing 0 mol%, 20 mol% and 40 mol% cholesterol. The free energy barriers for both cholesterol and DPPC flip-flop increase as the cholesterol concentration increases, due to increased order in the bilayer. For cholesterol, the free energy barrier for desorption increases as cholesterol concentration increases, indicating cholesterol prefers more ordered environments. In contrast DPPC prefers less ordered bilayers, as the barrier for DPPC desorption decreases dramatically as cholesterol concentration increases.

321-Pos Using Ethanol Molecules to disrupt DPPC/Ergosterol Interactions in Lipid Bilayers

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Board B154

Cholesterol, the main sterol component in higher eukaryotes, has a counterpart sterol, ergosterol, that is found in lower eukaryotes (such as yeast). As with cholesterol, ergosterol molecules are

important in maintaining the fluidity of plasma membranes and it is interesting to examine changes in the lipid/ergosterol phase diagram resulting from an ergosterol concentration change. Formation of an intedigitated phase, characterized by intertwining of lipid molecules from opposing bilayer leaflets, may play a role in "stuck fermentations". A stuck fermentation is the wine industry term for a premature stop of ethanol production due to unknown yeast metabolic changes. Yeast membranes consist of 10–25 mol% ergosterol and a wine fermentation can reach ethanol concentrations of 14 vol%. An experimental study found that at an enological relevant temperature and ~12–16% ethanol, interdigitation is visible between leaflets that contain ~10–25 mol% ergosterol and hence the induction of this phase may lead to reduced yeast productivity [1]. In this study, we use atomistic Molecular Dynamics simulations to examine the mechanical properties of lipid bilayers that contain ergosterol concentrations of 10, 20, 25 mol% with ethanol concentrations of 0, 10, 15 vol%. We calculated how the size of ergosterol clusters and the molecule MSD changes for several sterol/ethanol concentrations. We also examined how the equilibrated ethanol molecule location near the bilayer interface varies with sterol/ethanol concentration.

References

[1]. Biophys. J. *89*: 2481–2493.

322-Pos Cholesterol Improves The Transfection Efficiency Of Lipoplexes By Increasing The Effective Membrane Charge Density

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Board B155

Motivated by its important role in lipid-mediated gene delivery, we have studied the effect of cholesterol on the transfection efficiency (TE) of cationic lipid-DNA (CL-DNA) complexes. Cationic liposomes typically used for gene delivery consist of a combination of neutral and cationic lipids, with the neutral lipid either DOPE or cholesterol. A successful *in vivo* liposome mixture seems to require cholesterol, although this phenomenon is not understood. We have investigated the effect of added cholesterol and structurally related molecules (β-estradiol, ergosterol, ergocalciferol, dihydroisoandrosterone and progesterone) in the low-transfecting regime of the DOPC-DOTAP system. TE increases by a factor of ten with the addition of only 15 % cholesterol, and further addition of cholesterol continues to increase TE. X-ray diffraction confirms that the lamellar structure is retained with addition of cholesterol and other sterols. Recent work in our group has identified the membrane charge density (σ) as a universal parameter for TE of lamellar, DOPC containing CL-DNA complexes [1–3], with TE following a universal bell-shaped curve as a function of σ. Theoretical calculations considering the headgroup area of cholesterol and thus counting for an increase in σ, when DOPC is replaced by cholesterol, show that TE strongly deviates from the TE universal curve. However, experimental determination of σ via X-ray diffraction shows full agreement with the TE universal curve demonstrating that the real σ is higher as predicted, therefore the effective headgroup area of cholesterol is lower as expected by theory, suggesting that cholesterol is inserted deep into lipid bilayer partially hidden by neighboring lipids.

Funding by NIH GM-59288 and NSF DMR-0503347.

References

[1]. A.J. Lin et al, *Biophys. J.*, 2003
[2]. K. Ewert et al, *J. Med. Chem.*, 2002
[3]. A. Ahmad et al., *J. Gene Med.,* 2005

323-Pos Effect Of Cholesterol In The Solubilization Process By beta-D-fructofuranosyl-6-O-myristyl-alfa-D-glucopyranoside (MMS)

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Board B156

The solubilization of liposomes in the presence of amphiphillic compounds (the process involving transformation from lamellar structures to mixed micelles) has been usually described with the three stage model. In the first stage, at surfactant concentrations below its cmc, the presence of surfactant molecules in the vesicle or liposome induces several changes on its properties (mainly size increase observable turbidity or by light scattering), when the bilayer is saturated, the presence of mixed structures is observed (micelles formed by lipid and surfactant) in coexistence with lamellar structures (open or closed bilayers). A decrease in turbidity is observed upon further increase in surfactant concentrations, consistent with the complete solubilization of bilayers (remaining only mixed micelles in the system).

We studied the effect of the presence of cholesterol on the solubilization process by the sucrose monoester of myristic acid, beta-D-Fructofuranosyl-6-O-myristyl-alfa-D-Glucopyranoside (MMS) in different liposomes. We used LAURDAN (a membrane probe that gives information about the water content in the bilayer that is related with the membrane fluidity). Laurdan generalized Polarization (GP) measurements were performed in the cuvette and in the 2-photon microscope to follow the solubilization by MMS on POPC and DODAC liposomes containing different amounts of cholesterol.

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324-Pos The Influence of Environmental Conditions, Lipid Composition, and Phase Behavior on the Origin of Cell Membranes

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At some point in life's development, membranes formed, providing barriers between the environment and the interior of the 'cell'. This paper evaluates the research to date on the prebiotic origin of cell membranes and highlights possible areas of continuing study. A careful review of the literature uncovered unexpected factors that influence membrane evolution. The major stages in primitive membrane formation and the transition to contemporary cell membranes appear to require an exacting relationship between environmental conditions and amphiphile composition and phase behavior. Also, environmental and compositional requirements for individual stages are in some instances incompatible with one another, potentially stultifying the pathway to contemporary membranes. Previous studies in membrane evolution have noted the effects composition and environment have on membrane formation but the crucial dependence and interdependence on these two factors has not been emphasized. This review makes clear the need to focus future investigations away from proof-of-principle studies towards developing a better understanding of the roles that environmental factors and lipid composition and polymorphic phase behavior played in the origin and evolution of cell membranes.

325-Pos Measuring The Inter-Particle Interactions Of Lipid-Membrane Functionalized Microspheres

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The behavior of biomembrane-functionalized colloidal particles can reveal the physical properties of their biomolecular components, for example the charge states of lipids and their interactions with molecules in solution. Such microparticles are also potentially attractive tools for materials science: the facile tunability of lipid composition, the wide variety of lipids available with different electrical charges and headgroup chemistries, and the physical rearrangements that two-dimensional membrane fluidity makes possible all afford control of colloidal particle characteristics that is difficult or impossible to achieve by traditional techniques. An important metric of particle character is the pair interaction energy, U(r) – the energy as a function of inter-particle separation for a pair of identical particles separated by distance r. Despite indications of surprising and non-trivial inter-particle interactions from many-particle experiments, there have to date been no direct measurements of U(r) for lipid-membrane derivatized particles. Moreover, techniques to measure U(r) remain difficult and imprecise.

We have developed a new class of tunable optical line traps with which we directly measure U(r) for silica microspheres functionalized with fluid lipid bilayer membranes. The traps are made possible by control of the phase profile of the trapping laser beam, leading to direct control of the resulting one-dimensional intensity profile and trapping potential. Measurements of U(r) as a function of lipid composition, e.g. with varying concentrations of charged lipids such as phosphatidylserine and DOTAP, allows quantification of the particles' charge densities. The data reveal a surprising degree of charge regulation of the ionizable lipids and provide a quantitative characterization of these composite particles that will facilitate their use as new materials.

326-Pos Line Tensions, Correlation Lengths, And Critical Exponents In Lipid Membranes Near Critical Points

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Membranes containing ternary mixtures of cholesterol, a lipid with a high melting temperature, and a lipid with a low melting temperature can demix into two distinct two-dimensional liquid phases. At a miscibility critical point, the lipid compositions of the two membrane phases become equivalent. As the critical point is approached from high temperature, a membrane experiences increasing concentration fluctuations on length scales described by the correlation length. Similarly, as the critical point is approached from low temperature, the line tension at domain boundaries decreases to zero. We find that the temperature dependence of line tension and fluctuation correlation length in giant unilamellar vesicles fits the predictions of critical theory for a two-dimensional Ising model. Specifically, we measure correlation lengths between 0.5 and 4 microns with a power-law dependence on temperature. We measure values for line tension between 0.05 and 0.2 pN, and find that the decrease in line tension is linear with temperature near the critical point. We also measure the critical exponent for correlation length and obtain $1.22 \pm .27$, consistent with that predicted for the two-dimensional Ising model within experimental error. To our knowledge, liquid domains in membranes present the only system in which it is possible to image two-dimensional critical fluctuations in an optical microscope.

327-Pos Line Tension Measurements in Lipid Monolayers: Composition and Structure

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In the past decade intense interest has focused on the phase separation and lateral organization of two dimensional lipid systems. We describe a method for extracting the interfacial line tension between coexisting monolayer phases through direct observations of thermal fluctuations using fluorescence microscopy and digital image processing. Potential advantages of this method include the relatively few parameters required to determine the line tension and the ability to extend measurements to low magnitudes. Preliminary results demonstrate that the interfacial line tension calculated from the capillary wave spectrum is in good agreement with previous measurements employed using other experimental techniques [J. Phys Chem. B *111* pp11091–11094]. Experimentally, we extend these measurements to study the effect of cholesterol structure and monolayer composition on the line tension.

Theoretically, we are incorporating models which include electrostatic interactions into our work. We will briefly discuss how this technique potentially provides a new method to quantify protein-lipid interactions as well as capillary wave dynamics.

328-Pos Solute Partitioning At The Lipid-water Interface

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A variety of solvents are used in biology and biotechnology. For example, dimethyl sulfoxide (DMSO) is widely used as a cryoprotectant for mammalian cells during freezing. Brine solutions (highly concentrated salts) are used for food preservation, and low molecular weight polyethylene glycol (PEG) is used in most cosmetics and pharmaceuticals. These solvents and solutions have one thing in common: they strongly modify lipid interactions. By using a combination of small-angle scattering and density measurements, we determine how these modifications occur in the case of phosphatidylcholine (PC) lipids. We use the fact that PC membranes form multilamellar stacks with well-defined equilibrium spacings. The measured spacing represents the distance at which attractive forces between neighboring membranes are exactly balanced by repulsive ones. Any modification of the repeat spacing then is an indication of modified forces. We find that monovalent salt, DMSO, and low molecular weight PEG are all only partially incorporated in the interlamellar water spacing although they modify interlamellar forces in very different ways. Particularly puzzling is the observation that the measured DMSO is partially excluded from interlamellar water just like monovalent salt. Our density measurements show that suspended lipid vesicles float in DMSO solutions that are less dense than the lipid themselves. This indicates a net depletion of DMSO at the lipid-water interface despite its affinity to lipid headgroups. Implications of this project are important for understanding of how non-specific physical interactions in solutions and for applications in bioengineering, food processing, and drug formulations.

329-Pos Miscibility Of Inner Bacterial Membrane Components: Towards Insertion Of Lactose Permease Into Supported Planar Bilayers

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It is well documented that phosphoethanolamine plays a dual role in both activity and proper folding of transmembrane proteins. Lactose permease (LacY) of *Eschericha coli* has been reconstituted in functional state either into the native *E. coli* polar phospholipid extract membranes and into binary mixtures of their major constituents phosphatidylglycerol and phosphatidylehtanolamine. Here we have studied the mixing properties of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) at the air-water interface. The thermodynamic analysis suggests that interactions between the components are consistent with a lateral segregation of the molecules. In this work bilayers of the system that mimics the inner bacterial membrane (POPE:POPG, 3:1, mol/mol) were transferred onto a mica substrate and imaged with AFM under several conditions. Lateral phase segregation was demonstrated by topography images to be dependent on calcium presence (1). Then, incorporation attempts of LacY into supported planar bilayers were performed by incubation of purified LacY in dodecylmaltoside detergent with planar bilayers previously formed and extracting the excess of detergent (2).In another set of experiments we investigate the proximity of different POPE and POPG in the immediate vicinity (or annular region) of the lactose permease using fluorescence resonance energy transfer (FRET) techniques. After reconstituted into liposomes of different phospholipid composition, we measure the proximity between two single tryptophan mutants of LacY (W320 and 151W) and two phospholipids analogs of POPG and POPE: 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoglycerol, and 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoethanolamine, respectively. The results are interpreted as a consequence of lateral compressibility and mixing properties of these phospholipids (3).

References

1. S.Merino, Ò.Domènech, M. T. Montero, J. Hernandez-Borrell, (2005) Langmuir *21*, 4642–4647.
2. P.E. Milhiet, F. Gubellini, A. Berquand, P. Dosset, J.L: Rigaud, C. Le Grimellec, D. Levy (2006) Biophys. J *91*, 3268–3275
3. O. Domenech, J. Ignes-Mullol, M.T. Montero, J. Hernandez-Borrell (2007) J. Phys. Chem. B, *111*, 37, 10946–10951.

330-Pos Studying of Heterogeneous Langmuir-Blodgett Films from Alveolar Surfactants

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Our descriptions of hydrophobic interactions assumed an essential role of the interfacial/surface hetergeneity of interacting particles. As a crusial point was a suggestion that a descending branch of the surface domain size distribution on heterogeneous interface might be of the Poisson-type or the log-normal, but not the Gaussian one. This hypothesis was experimentally investigated in our study of heterogeneous Langmuir-Blodgett films (LB) of DPPC obtained from (mice) alveolar surfactants. *Sample preparation*. 19 μL/1 mM Dipalmitoylphosphatidylcholine (DPPC) (95%) and 1 μL/1 mM nitrobenzoxadiazol-labelled phosphatidylcholine (NBD-PC) (5%) were spread using the Langmuir -Blodgett technique. The Teflon ribbon trough S (Kibron Inc.) was used to compress at a rate of 2.00 X2/chain/min to the desired surface pressure. A deposit was made on glass or mica at a rate of 10mm/min. Doubly distilled H_2O was used as the subphase at ambient temperature. For fluorescence work, deposits were made onto 12 mm diameter glass coverslips (Fisher Scientific Co.) which had been rinsed with chloroform:methanol (3:1) after a 24 hour soak in sulfuric acid and ddH_2O. The deposits were examined by *fluorescence microscopy, atomic force microscopy, and goniometric* contact angle measurements.

The experimental data verifies the hypothesis of the character of the heterogeneity size distributions and lends credence to the predictions advanced in our theoretical works This has led to descriptions of attractive hydrophobic interactions, aggregating of biocolloids, and kinetic and static properties of boundary layers [1–3].

331-Pos Molecular Self-Assembly On Supported Planar Lipid Bilayers: Role Of Lipid Chemistry On The Formation Of J-Aggregates

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The self-assembly of dye molecules into so-called J-aggregate structures is characterized by unique spectroscopic signatures, signifying a specific two- or three-dimensional arrangement of the individual constituent molecules. These signatures have been exploited to report on the pH of intracellular organelles or compartments. We have used correlated confocal fluorescence - atomic force microscopy (AFM) and visible-light spectroscopy to investigate the self-assembly of pseudo-isocyanine dyes (PIC) on supported lipid bilayers. PIC aggregation on the bilayers was dictated by the ionic strength of the environment and lipid headgroup character. While zwitterionic phosphatidylcholine and sphingomyelin, along with phosphatidylethanolamine, were found to be reliable supports for J-aggregate growth, lipids with charged headgroups such as phosphatic acid and phosphatidylglycerol do not support the formation of these structures. Furthermore the fluidity of the bilayer dictated the emission wavelength and yield of the J-aggregate fluorescence. The growth of highly ordered fluorescent J-aggregates occurred preferentially on gel-phase domains, whereas the fluid-phase regions supports weakly fluorescent aggregates with different emission characteristics. These results suggest that assembly of PIC molecules into J-aggregate structures is facilitated by cation-π interactions between the positively charged lipid headgroups and the quinoline groups of PIC but that there is also a need for a specific spatial registry within the lipid bilayer itself that facilitates this stabilization.

332-Pos Hop Diffusion in Planar Model Membranes with Micron-size Compartments Using Quantum Dot-Conjugated Membrane Probes

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High-speed tracking experiments have revealed that lipids and membrane proteins of the plasma membrane show hop diffusion. This peculiar diffusion process has been interpreted in terms of fence-like diffusion barriers based on membrane proteins bound to filaments of the underlying cytoskeleton (picket-fence model), though the exact structural organization of these fences remains a topic of open debate. Here we report on a planar model membrane platform based on polymer-supported phospholipid bilayers where the diffusion fences are entirely protein-free, but still exhibit interesting parallels to their cellular counterparts. Unlike in cells, the average compartment size in our model system is above the diffraction limit of optical microscopy and can be easily determined using epifluorescence microscopy. Using wide-field single molecule fluorescence microscopy, long-term tracking experiments of photostable quantum dot-conjugated membrane probes are presented, which verify the occurrence of hop diffusion in these planar model membranes. The average size of fenced corrals of 1–2μm^2 determined from these tracking experiments agrees well with the average compartment size obtained by epifluorescence microscopy. The current study is particularly attractive because it provides, for the first time, a cell-free experimental platform for the study of hop diffusion based on easily observable diffusion compartments where important experimental parameters (e. g., the fence thickness and

density) can be controlled quite accurately. Corresponding experiments are discussed.

333-Pos Bending Membranes on Demand: Fluid Phospholipid Bilayers on Topographically Deformable Substrates

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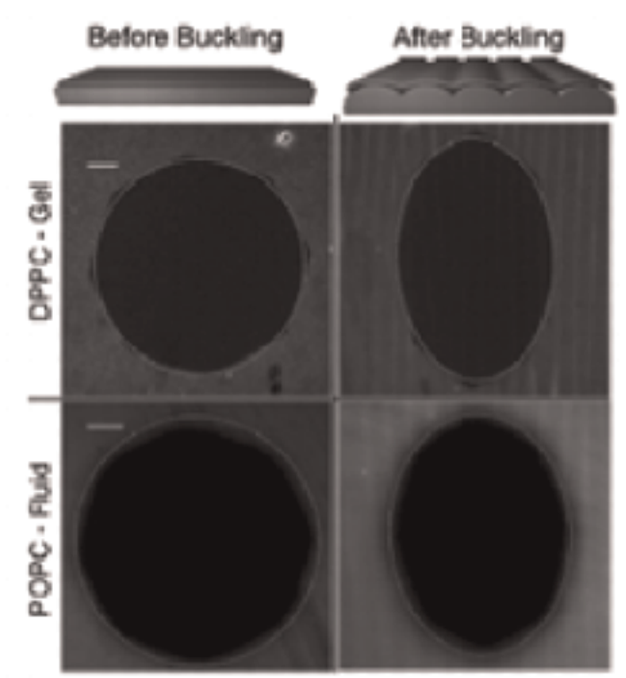
Living organisms have a dynamic scaffolding that can vary their membrane curvature, allowing a chemical-mechanical control of spatial organization of molecules and corresponding biological functions. Here we demonstrate that interfacing single phospholipid bilayers with deformable, oxidized PDMS elastomers in water allows us to create model membranes with complex three-dimensional topographies. Real-time variations in substrate topography trigger spatially patterned mesoscale reorganization of the bilayer trigger spatially patterned mesoscale accompanied by curvature-dependent molecular reorganizations. This ability to dynamically impose curvatures on supported bilayers and the ensuing re-equilibration promises fundamental biophysical investigations of curvature-induced functional reorganizations in membranes in a massively parallel manner. Beyond biophysics, these complex model membranes may also provide a generic means to create sustained molecular gradients and carry out spatial separation of membrane-compatible amphiphiles.



334-Pos Non-equilibrium Thermodynamics of Substrate-supported Lipid Bilayers from Conventional Calorimetry Experiments

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Traditionally, phase properties of lipid bilayers are derived from calorimetry measurements that are analyzed on assumptions of equilibrium thermodynamics. More recently, non-equlibrium thermodynamics of lipid bilayers has been assessed by monitoring kinetics of the heat flux followed a sudden pressure jump; the method has been called pressure perturbation calorimetry. The latter method provides the data on the relaxation times observed after the pressure jumps carried out at constant sample temperature. Such measurements require fast and sophisticated temperature control system while pressure jumps could cause formation of microbubbles (foaming) in bilayer samples. Here we describe non-equilibrium thermodynamic studies of lipid bilayers carried out at constant pressure with conventional differential scanning calorimeter (DSC). Our method is based on acquiring a series of DSC scans at variable temperature sweep rates and analyzing the data in terms of non-equilibrium thermodynamics based on heat dissipation theorem. The heat propagation through the sample cell and the intrinsic calorimeter time constants are also accounted for in the model. We show that this non-equilibrium DSC data are described by a Volterra integral equation that could be solved numerically. The method has been applied to study relaxation kinetics of unsupported lipid bilayers formed from DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) with and without cholesterol. The lipid relaxation parameters are compared with those of substrate-supported macroscopically-aligned bilayers formed inside ordered nanochannels of anodic aluminum oxide (AAO). Notably, substrate-supported nanotubular bilayers show existence of a slow-relaxing component that is likely to be associated with lipids in the direct contact with the oxide surface. Overall, we show that conventional DSC systems are suitable for studying relaxation kinetics of the heat flux to/from biological systems without subjecting those to sudden changes in thermodynamic conditions.

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335-Pos The pK's of Cardiolipins Are Altered by the Number and Position of Their Specific Fatty Acid Esters

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We have confirmed that the two phosphate groups of tetrastearoyl-cardiolipin have distinctly different pK values, with the second pK

being above neutrality. As a consequence, despite the appearance from the chemical structure, this phospholipid has an overall charge close to -1 at neutral pH. The cause for the high pK_2 is suggested to be a consequence of an H-bond network involving the two phosphate groups and the hydroxyl group on the C-2 of the glycerol that bridges the two phosphates in cardiolipin. Evidence for this has been provided by showing that deoxycardiolipin, having this central OH removed, no longer has a pK_2 above neutrality (Kates et al. (1993) Lipids, *28*, 877–882). We have extended these findings by examining the dependence of the titration of cardiolipin on the number and nature of the acyl chains. Our results demonstrate a strong difference in the titration of cardiolipin with its lysocardiolipin analog. The high pK_2, present in tetramyristoyl cardiolipin is missing in trimyristoyl cardiolipin. This and other results suggest that the formation of the hydrogen bond network at the surface of cardiolipin bilayers is strongly dependent on the interactions among molecules in the bilayer. The results may be relevant to the mitochondrial alterations observed in Barth's syndrome.

Membrane Physical Chemistry - II

336-Pos Carbonyl Configuration And Hydration Of Curved And Ripples States Of Lipid Interphases

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The L_β and the L_α phases of phosphatidylcholine are characterized by the levels of hydration. The P_β phase is assumed to have an intermediate hydration state between the L_β (5–7 water molecules per lipid) and the L_α phase (20 water molecules) and has been related with the appearance of ripples. In this regard, defective packing has been detected as a consequence of the coexistence of gel and fluid domains.

Carbonyls in the ester union of the phospholipids are distributed in two populations: one of them normal to the bilayer plane and the other parallel to it. The first one is in contact with water and hence, the frequency corresponding to its stretching mode is shifted to lower values with respect to the low hydrated population, which in turn, falls very near to that corresponding to dried phospholipids.

In this paper, we show by FTIR analysis that the extents of hydration of the ripples correspond with a clear separation of the center bands corresponding to the two populations. When temperature is decreased and the gel planar phase is attained the difference falls to values that makes impossible to distinguish between the two populations by deconvolution analysis.

Compounds that avoid the pretransition produces a shift in the carbonyl population congruent with the disappearance of the ripples phase. In this condition, when an osmotic shock is applied, the separation of the bands returns to those observed in ripples. It is concluded that both spontaneous and induced curvature induces defects at the interphase as consequence of the fluctuations in the orientation and hydration of the carbonyl groups at the water - hydrocarbon interface. This interpretation is compatible with reported values of water penetration beyond that plane.

337-Pos The Kinetics Of Receptor-mediated Virus Adsorption And Cytoplasmic Transport

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We derive the equations that describe adsorption of diffusing particles onto a surface followed by additional surface kinetic steps before being transported across the interface. Multistage surface kinetics occurs during membrane protein insertion, cell signaling, and the infection of cells by virus particles. For example, after nonspecific binding, additional kinetic steps, such as binding of receptors and coreceptors, must occur before virus particle fusion can occur. We couple the diffusion of particles in the bulk phase with the surface kinetics and derive an effective, integro-differential boundary condition that contains a memory kernel describing the delay induced by the surface reactions. This boundary condition takes the form of a singular perturbation problem in the limit where particle-surface interactions are short-ranged. Moreover, depending on the surface kinetics, the delay kernel induces a nonmonotonic, transient replenishment of the bulk particle concentration near the interface. Our approach generalizes earlier approaches to include surface kinetics, giving rise to qualitatively new behaviors. The transport of viral components to the nucleus will also be discussed.

338-Pos Membrane Interactions of Ternary Phospholipid/cholesterol Bilayers With Viscumin

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Small unilamellar vesicles (SUV) are produced by extrusion through polycarbonate membranes with 80 nm pores. Ternary mixtures of dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and cholesterol are used, yielding liposomes with final mean diameters of 144 nm (for DPPC/DOPC/cholesterol = 1:6:3 molar) or 166 nm (for DPPC/DOPC/cholesterol = 3:4:3). Viscumin, or mistletoe lectin, is a ribosome inactivating protein of class II. When viscumin is dissolved in phosphate buffer during formation of SUV, it distributes homogeneously inside and around the SUV with no elevated membrane adsorption, as proved by an enzyme linked immunosorbent assay.