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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Board B169

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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Board B170

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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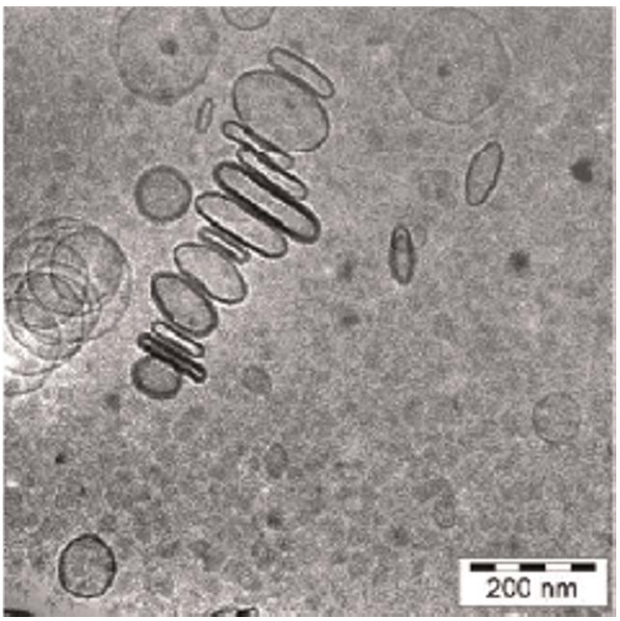
Small unilamellar vesicles (SUV) are produced by extrusion through polycarbonate membranes with 80 nm pores. Ternary mixtures of dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and cholesterol are used, yielding liposomes with final mean diameters of 144 nm (for DPPC/DOPC/cholesterol = 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.

Cryo transmission electron microscopy shows that with an increase of the saturated phospholipid DPPC the number of deformed discoidal SUV augments. These discoidal SUV tend to aggregate in piles, see figure. Aggregation is proportional to the number of viscumin molecules per vesicle for the same lipid mixture. For 30 mol% DPPC only about one vis-cumin molecule is needed to induce adherence of two vesicles. Aggregation is reversible as is disappears upon dilution and can also occur without proteins, e.g. for membranes with DSPC/cholesterol = 6:4. It is therefore assumed that aggregation is produced by hydrophobic interaction of the bilayer membrane.



339-Pos Volume Exclusion Effects of Polycations Near Charged Membranes

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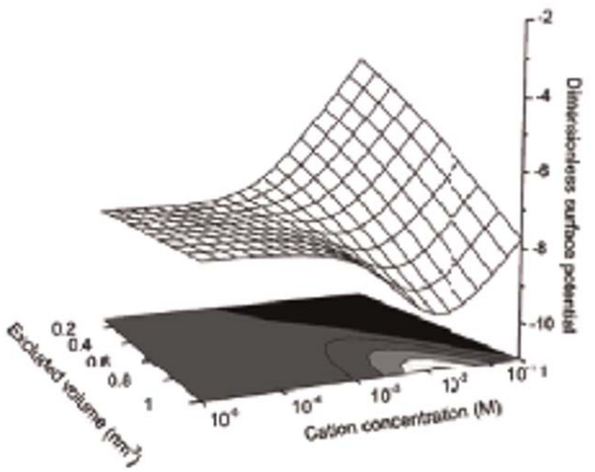
Board B172

The electrostatics of ions in solution is well described by the Poisson-Boltzmann theory in simple cases, but the discrepancy with the reality grows as ion size and valence increase. As an example, Monte Carlo simulations of a 3:1 finite-size electrolyte near a negatively-charged wall demonstrate a sign inversion of the diffuse potential non-predicted by the Poisson-Boltzmann theory. By incorporating the mixing entropy of the solvent into the chemical potential of each ion species, we arrive at a generalized Poisson-Fermi equation comprising the excluded volume of ions. This simple model predicts the overcharging of a typical anionic lipid membrane by large monovalent cations (Figure). Electrophoretic measurements performed on phosphatidic acid (DOPA) liposomes with polyamine cations reveal a damping of the screening effect consistently with the Poisson-Fermi paradigm. Ion adsorption mechanisms will be added to the model to account for the adsorption mechanisms will be condensation effects.

Figure. Calculated dimensionless surface potential of a negatively-charged wall in presence of a 1:1 electrolyte with varying excluded volumes for the cations. All other ions have a fixed excluded volume of 0.15 nm^3. The wall charge density is -0.2 $C.m^{-2}$, and the electrolyte contains 10 mM of a 1:1 buffer.



340-Pos Effects of Ceramide on Sphingomyelin Membranes: Increased Thermal Stability and Chain Order

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Board B173

Sphingomyelin, a major component of mammalian cell plasma membranes, is converted to ceramide during apoptosis. To investigate the structural changes to the membrane that accompany this conversion, we have used deuterium nuclear magnetic resonance (NMR) and differential calorimetry (DSC) to study multilamellar liposomes composed of *n*-palmitoyl sphingomyelin (SPM) and *n*-palmitoyl sphingosine (CER). For the NMR experiments, either SPM or CER was deuterium-labeled on the palmitoyl chain. Both NMR and DSC show that Cer markedly increases the onset temperature of SPM/CER gel to liquid crystalline melting, as well as the temperature of the liquidus. Both techniques also reveal that for CER concentrations of approximately 30% or less, there is evidence of a three-phase line at 39 +/− 2°C. At high temperatures, SPM and CER form a homogeneous liquid crystalline phase in which both sphingolipids' palmitoyl chains are observed to become slightly more conformationally ordered as the CER concentration is in-

creased. At physiological temperature, CER production in cell membranes is thus expected to increase the gel phase propensity of sphingolipid-rich regions of the plasma membrane.

341-Pos Small-angle X-ray Scattering and Molecular Dynamics Simulations of Amphiphilic Block Copolymer Association with Model Biomembranes

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Board B174

The use of amphiphilic block copolymers of PEO_n-PPO_m-PEO_n, so-called Pluronics or Poloxamers, or PEO_n-PPO_m as agents to modify cell membrane structure and function is an area of increasing research interest. Controversy remains, however, as to the means by which the tri-or diblock copolymer is incorporated into a lipid bilayer, particularly, how polymer architecture dictates its association and how this interaction influences membrane structure and properties. In order to gain a fuller understanding of polymer - membrane interactions, we report our recent efforts directed at developing a procedure to determine the one-dimensional electron-density profiles from freely suspended lipid (DMPC)-based bilayers containing these polymers using synchrotron small-angle X-ray scattering (SAXS). Interpretation of the experimental data is facilitated by coarse-grain molecular dynamics (MD) simulations which are used to predict the most favorable polymer conformational state upon association with the lipid bilayer. The determined 1-D electron distance distribution function encodes information on the structure of the lipid bilayer and the conformational state and projection of EO chains from the bilayer. These studies have shown that for both tri- and diblock copolymers the number of molecular repeat units in the PPO block is critical for determining the nature of polymer-membrane association . The nature of PPO integration in turn is an important factor that determines the conformational state of the associated PEO unit(s). For polymer architectures that integrate completely with the lipid bilayer a coiled conformational state of the PEO chains directed from the bilayer, produces steric pressure on the bilayer, causing a thinning of the membrane and leading to a rigid bilayer. The results of these studies and their fundamental significance to the application of these polymers in the amelioration of a variety of cellular injuries will be presented.

342-Pos The Investigation of Ionizing Radiation- Induced Damages on Liver Microsomal Membranes by FTIR Spectroscopy

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Board B175

The aim of the current study is to investigate the effect of ionizing radiation which is used in radiation therapy on rat liver microsomal membrane at molecular level. Fourier Transform Infrared (FTIR) spectroscopy was used for this purpose, which detects changes in molecular vibrations, providing information about the microenvironment of molecules. It also enables a correlation between chemical information and histological structures [1–3]. Sprague-Dawley rats were whole-body irradiated using Cobalt-60 irradiator at a single dose of 800 cGy, decapitated after 24 h and the microsomal membranes isolated from the livers of these rats were used for spectroscopic studies. The results revealed an increase in the concentration of unsaturated lipids in the treated samples which may be attributed to an increase in lipid peroxydationend products in the irradiated system. In addition, an increase in the concentration of phospholipids and fatty acids with an increase in lipid order and a decrease in membrane dynamics were observed. The radiation also caused an increase in the protein concentration in microsomal membranes. Furthermore radiation induced significant conformational changes in the protein structure. In conclusion, the whole body irradiation of rat with a single dose of 800 cGy leads to some significant changes in the structure, composition and function of the liver microsomal membranes.

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343-Pos Membrane remodeling by N-BAR domain proteins: Insights from multiscale simulation

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Board B176

Membrane remodeling can be examined at multiple length and timescales. Experiments naturally probe membrane remodeling on very long length and time-scales relative to the molecular level, and can observe both tubulation and vesiculation of liposomes due to N-BAR domain binding. We have recently employed large-scale atomistic level molecular dynamics (MD) simulation to examine the process of local membrane remodeling whereby a single or small number of N-BAR domains bend and distort a bilayer over time-scales of tens of nanoseconds. With these simulations, the process of local membrane remodeling can be examined at an atomistic-level of detail. However, connecting the observations made at the molecular scale (i.e., MD simulation), with the experimental results requires a multiscale simulation methodology whereby MD simulations are bridged with corresponding coarse-grained, and then

mesoscopic simulations, of N-BAR domain induced membrane remodeling.

We will present the results of our multiscale simulation study which indicates that membrane remodeling occurs via a molecular-scale local process, as well as with a mesoscopic collective effect. The collective effect arises due to the long-time average interaction of many N-BAR domains with the bilayer and with themselves. In particular, the spatial correlations of the N-BAR domains on the surface of the liposome can affect the process of remodeling. The results give some new insight into experimentally observed N-BAR domain induced membrane remodeling of liposomes.

344-Pos Controlling the Charge and Organization of Anionic Lipid Bilayers: Effect of Monovalent and Divalent Ions

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Board B177

Many physiological processes require local organization of membrane components, which could be facilitated by lipid de-mixing. Phospholipid bilayers, especially those containing anionic lipids, are strongly coupled to their solution environment, which allows ions to act as a control parameter for lipid phase separation. Using epi-fluorescence microscopy, we will show that the organization of membranes containing phosphatidic acid (PA) and phosphatidylcholine (PC) can be controlled by varying the monovalent/divalent ion ratio - a parameter that cells do control. Results will be compared to predictions from electrostatic theory. Additionally, we will show that PA/PC systems display both strong hysteresis and path dependence, which has interesting implications for local organization in cell membranes.

345-Pos The Role of Membrane Hydrocarbon Chain Composition in Interactions with Antihypertensive Drugs

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Board B178

Stressful life style of modern human societies leads to many diseases and one of the growing indispositions related to this fact is hypertension. Thus, there is a strong need for novel effective drugs. One of the new categories of antihypertensive drugs consists of AT_1 antagonists (SARTANs), whose action is based on blocking of the active site of the AT_1 receptor. The aim of the present study is to contribute to a basic understanding of the their molecular mode of action on membranes. We studied the influence of the SARTAN losartan on the global structure of phospholipid bilayers composed of pure dimyristoyl phosphatidylcholine (DMPC) and palmitoyl oleoyl phosphatidylcholine (POPC) applying synchrotron small-angle x-ray scattering. Additionally, we also studied influences on the binary mixtures of DMPC/cholesterol and POPC/cholesterol. The observed effects were primarily related to bilayer interactions, whereas the membrane thickness remained largely unaffected. Losartan led to a complete loss of positional correlations between adjacent bilayers for all single component model membranes. This can be explained by the negative surface charged conferred to the bilayers upon losartan insertion. The effect was however, counterbalanced upon the addition of cholesterol. Both, POPC and DMPC bilayers exhibited no positional correlations up to 5 mol% cholesterol, respectively. However, only POPC remained uncorrelated at 20 mol% and above, while DMPC/cholesterol bilayers exhibited multilamellar vesicles, i.e. a reentrant transition of the positionally uncorrelated bilayers into correlated ones. Our results may be understood in view of the different hydrocarbon chain packing densities in saturated versus unsaturated bilayers and their respective affinities to interaction with cholesterol. This shows that the insertion of losartan into the membrane may be overridden by a tighter bilayer interface and emphasizes the role of hydrocarbon chain composition in its mode of action.

346-Pos Uptake & Release Protocol for Assessing Membrane Binding and Permeation

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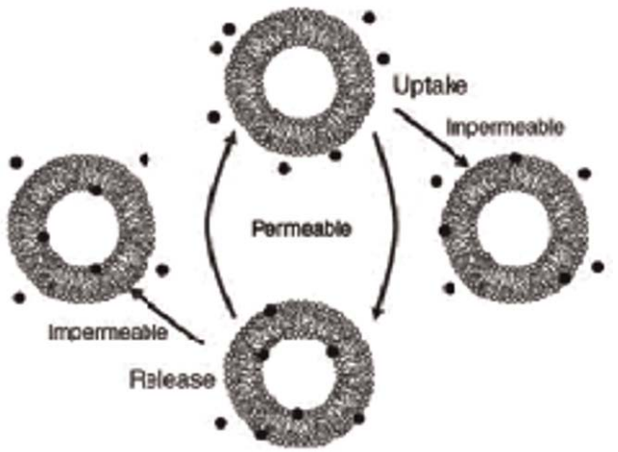
The activity of many biomolecules and drugs crucially depends on whether they bind to biological membranes and whether they translocate to the opposite lipid leaflet and trans aqueous compartment. A general strategy to measure membrane binding and permeation is the uptake & release assay, for which the detailed protocol and a script for data evaluation are now available from Nature Protocols *2*:695–704 (2007). /

The assay compares two apparent equilibrium situations established either by the addition or by the extraction of the solute of interest. Only solutes that permeate the membrane sufficiently fast do not show any dependence on the history of sample preparation. This strategy can be pursued for virtually all membrane-binding solutes using any method suitable for detecting binding; isothermal titration calorimetry and fluorescence spectroscopy have been used so far. Applications include the study of membrane binding and flip-flop of nonionic and charged solutes, the membrane insertion and translocation of insoluble compounds such as cholesterol or hydrophobic drugs, and the thermodynamic characterization of bilayer

curvature strain. The talk presents the strategy and gives an overview of the applications described so far.



347-Pos Charges in POPC Monolayers - A Temperature Dependant Monolayer Study

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Board B180

The surface of a bio-membrane is the first site of interaction for many bio-physico-chemical processes. The access to the surface, and the local structural modifications induced to the lipid bilayer upon interaction with an adsorbing guest molecule must be influenced significantly by the presence of charges, and local changes in surface charge density. Here we present preliminary results from an isothermal compression study of POPC monolayers into which charges were inoculated by addition of the ethylated lipid variety. The phase state will be discussed in view of its relevance for surface adsorption. The phospholipids examined in this study are: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (EPOPC).

Charge effects on POPC monolayers at the air-water interface were investigated by addition of different percentages of cationic EPOPC. These two phospholipids have the same molecular sequence apart from a slight difference in the headgroup region. The isotherms obtained for the mixtures are compared to pure POPC monolayer isotherms as the standard system.

Temperature dependence of isotherms for both pure POPC and the different monolayer compositions were examined at temperatures ranging from 5°C to 50°C. The π-A isotherms for POPC exposed a behavior which agrees well with established theoretical approaches for temperatures above 20°C: in that temperature region the surface pressure starts to increase earlier when the temperature is raised. However at temperatures lower than 20°C non-ideal behavior is observed in that surface pressure increases again upon decrease in temperature. The minimum in surface pressure as a function of temperature is seen around 20°C. The increase in monolayer's surface pressure upon introduction of a surface charge indicates repulsive interaction between the molecules. This might decrease stability of film thus, induce curvature frustration and might destabilize a bilayer formed from such a mixture.

348-Pos Effect Of Lipid Composition On The Vesosome

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Board B181

An optimal drug delivery vehicle should circulate long enough to reach the site of illness or disease, possess a large drug loading capacity and be able to deliver its contents at a rate appropriate for maximum therapeutic benefit at the site of interest. The liposome - a closed, spherical lipid bilayer encapsulating an aqueous core - has been extensively examined as a drug delivery vehicle over the last several decades. Even with recent advances, liposomes continue to be plagued by issues with drug retention, delivery and toxicity. A new liposome-based drug delivery carrier designed to address these problems is the vesosome - a large lipid bilayer enclosing many small liposomes. This structure offers a second layer of protection for its contents and also allows for variation of internal liposomal lipid composition to accommodate multiple drugs. In addition, internal compartmentalization permits customization of separate aqueous environments for therapeutics and gold nanoshells, which may potentially be used as release triggers for the vesosome. The attractive innovation of the vesosome is its ability to achieve a single site, single dose, multiple component drug treatment. The structure, drug retention and drug release of several vesosome lipid compositions were compared. Vesosome structure was examined using freeze-fracture transmission electron microscopy. Drug retention and release profiles in phospholipase A_2 and blood plasma were determined using a combination of fluorescence spectroscopy and ^{19}F-NMR. Results indicate that release time is affected by changes in the lipid composition of its internal liposomes while overall vesosomal structure or behavior is not. Compared to liposomes in similar media, all vesosomal formulations exhibit increased drug retention.

349-Pos Effect of Curvature on Hydration of Stratum Corneum Lipid Membrane

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Board B182

Stratum corneum (SC) is the outermost layer of the skin that functions as the main permeability barrier. Lipid bilayers found in the SC are believed be important in determining the permeation properties of the skin. The effect of curvature on the hydration level of SC lipid membranes is studied by using a polarity sensitive fluorescence probe, Laurdan. Lipid membranes made from equimolar ratio of brain ceramide, palmitic acid and cholesterol are used as model SC system. The curvature of lipid bilayers is controlled by varying the size of lipid vesicles via extrusion process. The size distribution of extruded lipid vesicles is characterized by dynamic light scattering and cryo-fracture scanning electron microscopy. Fluorescence emission of Laurdan incorporated in the extruded vesicles indicates that high curvature imposed by smaller vesicles lead to higher level of hydration in SC lipid membranes. Phospholipid vesicles also show the similar trends in their hydration level as their size are varied. The permeability of the SC lipid membranes with varying curvature is also investigated.

350-Pos The Use of Steady-State FRET to Determine Phase Boundaries in 3-component Lipid Bilayer Mixtures

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Board B183

Over a period of several years, a number of investigators have determined the phase diagram for the 3-component mixture DSPC/DOPC/cholesterol, at 23C. Fluorescence microscopy imaging of GUVs provided crucial data for interpreting the various regions that have coexisting phases. When DOPC–a rare lipid in animal cell plasma membranes–is replaced by the naturally abundant lipid POPC, the fluorescence microscopy images of GUVs still reveal a region of coexisting {La + Lb} phases, but do not show any macroscopic domains of {La + Lo} coexistence. One conclusion might be that the 3-component mixture DSPC/POPC/chol does not have coexistence of separate phases of {La + Lo}, but perhaps only nonrandom mixing of the components. However, we found that FRET experiments using two donor/acceptor pairs, DHE/BoDIPY-PC and BoDIPY-PC/C18:2-DiI, over the entire composition space of DSPC/POPC/chol at 23C are best interpreted by a model of probe molecules partitioning between distinct phases, with different phase regions separated by well-defined phase boundaries.

351-Pos Electroformation of Mixed Phospholipid Vesicles

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Board B184

Giant phospholipid vesicles are model systems for the study of biological membranes. The electroformation method (1) produces giant unilamellar vesicles (GUVs). These vesicles are easy to manipulate and are currently used in order to study the mechanical properties of membranes with different techniques. We have studied the electroformation kinetics of vesicles formed with mixtures of two phospholipids: SOPC (neutral) and SOPS (charged). By changing the relative composition of the membrane, the charge density is modified. This system has been chosen because the vesicles obtained by hydrating phospholipid films of these mixtures have characteristic shapes depending on the relative compositions of the membrane (2). Our experimental results show that the electroformation kinetics depend on the charge content of the system. We discuss the physical parameters relevant for the experiments that a theory of the electroformation process should take into account.

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352-Pos Sterol Superlattice Formation Affects Antioxidant Potency And Can Be Used To Assess The Possible Adverse Effects Of Antioxidants

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Board B185

We have developed a fluorescence assay to examine how sterol lateral organization affects antioxidant potency. This information is used to evaluate the adverse effects of lipid-soluble antioxidants seen in recent clinical studies. The connection between lateral organization and antioxidant potency is based on the sterol superlattice theory. This theory proposes that membrane properties vary with sterol content in an alternating manner, showing a biphasic change at critical sterol mole fractions (C_r) for maximal superlattice formation. Using dehydroergosterol (DHE), we have measured the duration of the lag phase (τ) produced during free radical-induced sterol oxidation at different antioxidant doses and different sterol mole fractions in fluid DHE/DMPC unilamellar vesicles. Ascorbyl palmitate (lipid-soluble vitamin C) always generates a longer τ[[Unsupported Character - ]] value than ascorbic acid (water-soluble). The ascorbic acid-induced τ value varies with sterol mol% in a biphasic manner, showing a minimum at ~C_r, indicating that sterol superlattice formation persists in the ascorbic acid concentration range examined (0–120 μM) and that sterol superlattice formation affects antioxidant potency. In sharp contrast, the biphasic change in τ at C_r is observed only at low doses of ascorbyl palmitate (<15 μM). This result suggests that while ascorbyl palmitate would be a more efficient antioxidant than its water-soluble counterpart as judged by the τ value, ascorbyl palmitate can easily perturb sterol lateral organization due to its insertion into membrane bilayers. Because sterol lateral organization is of vital

importance in cell membrane functions, our finding partially explains why a lipid-soluble antioxidant may impose detrimental effects. The threshold antioxidant concentration to abolish a biphasic change in τ at C_r may vary with antioxidant; this method could be employed to assess the potential adverse effects of other lipid-soluble antioxidants.

353-Pos Effects of PAMAM Dendrimers on DPPC and POPG Bilayer Membranes: Molecular Dynamics Simulation Study

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Board B186

We have performed full atomistic molecular dynamics simulations for studying the interaction between ethylenediamine (EDA) core polyamidoamine (PAMAM) dendrimers (Generation 3) and various biological membranes such as neutral dipalmitoylphosphatidylcholine (DPPC) and negatively charged palmitoyloleoylphosphatidylglycerol (POPG). Since the PAMAM dendrimer is positively charged at neutral pH, it strongly adsorbs on the surface of the POPG membrane, affecting the structural and dynamical behavior of the membrane. We discuss these effects of dendrimers on biological membranes by presenting various structural and dynamical properties such as area per lipid, membrane thickness, density profile, order parameter, radius of gyration, and self-diffusion.

354-Pos Binding of Alkyl Ether substituted $Ru(phen)_2(dppz)$ Derivatives to Liposome Membranes

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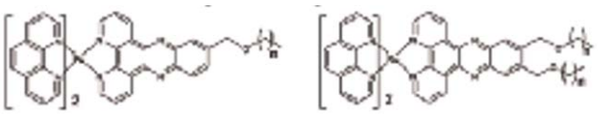
Board B187

Ruthenium complexes with dipyridophenazine ligands have been frequently studied for their DNA binding properties during the last decades. These complexes display no luminescence in aqueous buffer but show a strong luminescence increase in the presence of DNA, the "light switch effect". This effect is also seen when ruthenium complexes bind to a phospholipid membrane.

In this work dipyridophenazine derivatives with alkyl ether chains of different lengths (Fig. 1) are investigated by their different interactions with a membrane bilayer. Phospholipid vesicles, liposomes, with a net negative charge and a diameter of 100 nm is used. Steady state luminescence studies and excited state lifetime measurements compare the binding and insertion depth between different complexes. Further, the insertion and orientation of the complex in the liposome is quantitatively investigated by linear dichroism where retinoic acid is used as an internal membrane probe of orientation. Also the possibility to use these ruthenium complexes to functionalize membranes by binding DNA to the substrate is studied. Additionally, the ability for electron transfer from the charge separated excited state to a membrane embedded acceptor is investigated.

Figure 1. Molecular structures of $Ru(phen)_2(dppz)$ derivatives.



355-Pos The Effects of Radioprotectant Amifostine on Zwitterionic Dipalmitoyl Phosphatidylcholine (DPPC) Membranes

Gulgun Cakmak, Feride Severcan

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Amifostine (WR-2721), an aminothiol, is the only approved radioprotective agent by the Food and Drug Administration (FDA) to reduce cytotoxic effects of radiation used in radiotherapy. Research on the effects of amifostine is mainly on clinical level, whilst the molecular effect of amifostine on the biological membranes are unknown, yet. Since biological membranes have very complex structures, the examination and interpretation of the interaction between molecules with these membranes are very difficult. For this reason, in the current study we used a zwitterionic model membrane which has similar characteristics with biological membranes [1, 2]. Interactions of amifostine with zwitterionic dipalmitoyl phosphatidylcholine (DPPC) multilamelllar liposomes (MLVs) were investigated as a function of temperature and amifostine concentration (1–24 mol%) by using two non-invasive techniques, namely Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The results revealed that, amifostine did not change the main phase transition temperature and the shape of phase transition curve indication the prefential location of amifostine near to aqueous site of the membrane. Whilst the investigation of the CH_2 and CH_3 antisymmetric, C=O and PO_2^- stretching modes in FTIR spectra showed that amifostine changes physical properties of the DPPC bilayers. For instance, amifostine decreased the order of the DPPC MLVs in the liquid crystalline phase and decreased the dynamics of the DPPC MLVs both in the gel and in the liquid crystalline phases. Amifostine also caused significant decrease in the frequency of the C=O stretching and PO_2^- antisymmetric double bond stretching bands, which indicates strong hydrogen bonding around the head groups of the liposomes.

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356-Pos Characterization of Acyl Chain Dynamics, Packing, and Vesicle Morphology of 18:1 BMP: An NMR and EPR Investigation

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Solid-state 31-Phosphorus NMR and doxyl-spin label EPR, along with dynamic light scattering (DLS), are used to investigate the effects that bis(monoacylglycero)phosphate (BMP) has upon the vesicle morphology of POPC. BMP is a phospholipid that is enriched in late intraendosomal vesicles, and is thought to be involved in lipid and vesicle trafficking. The structure of BMP is unique, with a single phosphate group, two glycerol backbones and singe acyl chains at the 3 and 1′ glycerol hydroxide residues. Combined, the NMR and DLS results indicate that as little as 1% BMP causes the formation of small vesicles, d $<$ 100 nm. These results are consistent with the biological finding that the induction of BMP formation in early endosomes correlates with the budding of smaller intraendosomal vesicles [1]. The morphology of 100% BMP vesicles were also investigated with 31-P NMR and DLS. Detergent solubilization of BMP with SDS and OG was followed by EPR spectroscopy of BMP mixed with 1% doxyl-labeled PC lipids. Detergent solubilization of BMP proceeds in a similar manner to that of POPC vesicles, indicating that the packing of BMP molecules is similar to that of POPC.

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357-Pos A Free Energy Model For The Observed Morphologies Of The Crista Membrane Of Mitochondria

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Electron tomograms have revealed that in normal mitochondria the crista membrane contains both tubular and flat lamellar components. Using a simplified model, we have been able to predict the conditions under which the observed morphologies can be obtained by minimizing the system's free energy. The model predicts that the tubular structures are stabilized by tensile forces of the order of 10 pN, comparable to those typical of motor proteins. The model also predicts reasonable values for the pressure difference across the crista membrane and its surface tension. Currently we are investigating whether our free energy model can predict a more recently observed morphology*, a transformation of the mitochondrial inner membrane into multiple vesicular matrix compartments observed during programmed cell death (apoptosis) in HeLa cells treated with etoposide. We plan to report these results as well.

*Sun, M.G., Williams, J., Munoz-Pinedo, C., Perkins, G.A., Brown, J.M., Ellisman, M.H., Green, D.R., and Frey, T.G. Nat Cell Biol *9*, 1057–1072 (2007)

358-Pos Nanosecond Biomolecular Electrosurgery - Mechanism of Pore Formation in Lipid Bilayers

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Molecular dynamics simulations of phospholipid bilayers in high electric fields reveal details of electroporation kinetics and dynamics not directly accessible by experiment. The mechanism of pore formation suggested by these simulations_extension of stochastic water defects into bilayer-spanning water channels which are lined and stabilized by the diffusive migration of phospholipid head groups along the incipient nanometer-diameter pore walls within nanoseconds_is consistent with observations of artificial membranes and living cells in electric fields, and with continuum physical and electrostatic models. Water defect propagation into the bilayer interior is enhanced by the alignment of interfacial water dipoles in the applied electric field and by the degree of saturation of the lipid hydrocarbon tails, and is relatively insensitive to the net charge on the head group.

Extensive simulations were performed to understand the mechanism of pore formation, dependencies on system composition, and pore formation thresholds, and toexplain experimental observations of differences in pores formed at the anode- and cathode-facing poles of artificial vesicles and living cells. The breakdown potential and associated threshold field for which a water defect becomes a hydrophilic pore is correlated with the hydrocarbon thickness of the bilayer and is weakly dependent on lipid area, temperature, location and number of unsaturated bonds, and orientation of the lipid headgroups. This implies that pore formation is limited by free energy rather than kinetic considerations. Still, the precise relationship between the free energy barrier of pore formation and the electric field requires further investigation.

359-Pos Dynamic Phase Microscopy: Is A 'dialogue' With The Cell Possible?

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The metabolic component of refractivity is considered a quantitative parameter of cellular 'vitality' or as a factor supplementary to the Hamaguchi's 'signature of life'. It is shown that the method of dynamic phase microscopy provides real-time information about intracellular processes as local changes in the optical path differ-

ence. The measurements of single cells and organelles were performed with high spatial (100 nm) and temporal (1 ms) resolutions with the original coherent phase microscope 'Airyscan". Data analysis yielded the corresponding spatial-temporal and spectral 'portraits', the dynamic topograms and the low-frequency audio signals. Presented are the examples of cooperative processes in human cells as local fluctuations accompanying the pre-ribosome synthesis in response to cytotoxic drugs and as ribosomal 'voices' in cyanobacteria. We demonstrate the possibility of localizing 'signals' and obtaining information regarding the signal-structure functions in mitochondria, cyanobacteria *Anabaena* and the nucleoli. This approach enables a 'dialogue' with a cell by registering the response of biological objects to external milieu in real time. The problems of cell 'language', 'dialogue', and biophysical interpretation of signals are discussed. Also, I analyze the dependence of refractivity on metabolic states, in particular, the contribution of the bound water molecules in protein hydration shells.

360-Pos Probing Ciprofloxacin - Lipid Interaction By Atr-ftir And ^{31}P Nmr Spectroscopy

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The interactions between the fluoroquinolone ciprofloxacin (CIP) and lipids have been described in the literature. We previously showed that the ability of CIP to induce disorder and modify the orientation of the acyl chains is related to its propensity to be expelled from a monolayer upon compression (*Fa, Bensikaddour et al., submitted).*

Here, we investigated CIP effects on the transition temperature (Tm) of lipids and on the mobility of phosphate head groups using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) and ^{31}P Nuclear Magnetic Resonance (NMR). The selected phospholipids were DPPC and DPPG. ATR-FTIR experiments showed that CIP had no effect on the Tm of DPPC but increased the order of the acyl chains both below and above this temperature. In contrast, with DPPG, CIP induced a marked broadening effect on the transition with a decrease of the acyl chain order below its Tm and an increase above this temperature. Furthermore, ^{31}P NMR data showed that CIP bound to lipid model membranes and decreased the mobility of phospholipid head groups. As compared to the control samples, the chemical shift anisotropy (Äó) values of DPPC:CIP (1:1, M:M) and DPPG:CIP (1:1, M:M) were respectively 5 and 9 ppm higher. Altogether, these data have demonstrated that the interactions of CIP with lipids depend markedly on the nature of their phosphate head groups and that the major effects of CIP on the bacterial membranes should be related to interaction with anionic lipid compounds

361-Pos Surface Characterization Of Phosphatidylethanolamine Containing Membranes

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In isolated forms, unsaturated PE′s stabilize in the inverted hexagonal H_{II}. In mixtures with PC′s the presence of PE determines the propensity of the membrane to abandon the bilayer structure.

PE′s have a very low hydration in comparison to PCs. This has been ascribed to the strong P - N interaction between lateral lipids in the solid lattice which, in turn, hinders the hydration of the phosphate. As the hydration of the lipids determines the packing, the polarization and the surface free energy of the interphase, the presence of PE may modulate the surface properties of lipid membranes in its interaction with additives, such as proteins. In fact, the effects of aqueous soluble proteins is the much lower on the surface pressure of PE monolayers in comparison to PC′s for similar hydrocarbon chains, as suggested by the lower cut off in the $\Delta\Pi$ vs Π curves.

FTIR analysis shows that the shift in the band of the antisymmetric stretching of the phosphates due to the hydration of the phosphate groups is much lower in PE′s in comparison to PC′s with similar chain length. This is congruent with a lower hydration and with the possibility that the P-N interaction still remains in fully hydrated lipids.

The rigidity at the head group level is demonstrated by the highest energy involved in the orientation of the phosphoethanolamine in comparison to phosphocholine dipoles. The lateral head group interactions are more important than hydration as a limiting factor for the reorientation of the polar heads. The decrease in hydration is congruent with the decrease in the area per lipid. In this condition, the dipole potential of PE is higher than in PCs, denoting that it is mainly determined by constitutive groups and not for water dipoles.

362-Pos Function and Organization of Mycobacterial Trehalose Dimycolate in Membranes

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Mycobacterial infections cause a high number of deaths worldwide because of its resistance against a large number of host defence peptides, which are active against other Gram-negative or -positive bacteria. One reason for this resistance is the unique structure of the mycobacterial cell well. Mycobacteria produce a thick mycolate-rich outer covering which functions as an exceptionally efficient barrier.

In this work we focused on the membrane forming properties of trehalose dimycolate (TDM) as one of the important components of the outer barrier. We reconstituted pure TDM layers and lipid mono- and bilayers consisting of PE:PG - as a model matrix - and TDM. We investigated the properties of these layers by means of film balance measurements, atomic force microscopy (AFM), and reconstitited planar lipid bilayers. TDM formed very stable monolayers and, when embedded into the lipid matrix, it self-aggregates into stable domains. Using force spectroscopy we could show that TDM layers have a higher mechanical stability as compared to pure phospholipid membranes. Furthermore, reconstituted TDM-containing membranes could not be permeabilized by the antimicrobial peptide LL32, which is a very active fragment of the human cathelicidin. In killing experiments we could show that LL32 is not active against mycobacteria from which TDM was purified. We propose that TDM contributes to the stability of the mycobacterial cell wall and strongly impairs the membrane permeabilization by host defence peptides.

363-Pos Glycolipid-derived Dehydration Resistance In Model Mycobacterium Tuberculosis Membranes

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The resurgence of tuberculosis in the developed world and its continued persistence in the developing world are well known public health concerns: roughly one-third of the human population is infected with Mycobacterium tuberculosis and each year two million people die as a result. The physical properties of the bacterium outer envelope are suspected to play a key role in its persistence. All mycobacteria, including Mycobacterium tuberculosis, have a dense outer membrane consisting of large fatty acids of which the glycolipid trehalose 6,6′ dimycolate (TDM) is a major component. Most studies of TDM and other envelope molecules, especially in recent years, have focused on the molecular biology of their expression and their interactions with host immune cells, leaving the physical properties of the envelope and the advantages it confers on pathogenic bacteria poorly delineated.

Given the ability of mycobacteria to withstand desiccation and the ability of soluble alpha, alpha-trehalose to protect proteins, cellular membranes, and whole organisms during desiccation, we hypothesized that TDM may alone be sufficient to confer upon its constituent membranes resistance to dehydration. To test this, we devised an experimental model that mimics the structure of mycobacterial cell envelopes, in which a TDM-rich, fluid outer leaflet is supported by an immobile hydrocarbon layer. We demonstrate, for the first time, the formation of two-dimensionally fluid TDM membranes, and find that they can be dehydrated and rehydrated without loss of membrane integrity or fluidity. More strikingly, this protection from dehydration extends to TDM-phospholipid mixtures in which TDM is a minority component, down to only 20 mol% TDM.

364-Pos New Insights into Surfactant Multilayer Formation. An AFM and Tof-SIMS Study

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Pulmonary surfactant is a complex mixture of lipids and proteins that forms a surface active film at the air-water interface of alveoli capable of reducing surface tension to near 0 mN/m. At the interface, lung surfactant has been presumed to exist as a monolayer, however, recent data, both from intact lung and from transferred films, have suggested that surface-associated multilayers form at high π values during expansion-contraction cycles (1–3). We investigated which components of surfactant are required for multilayer formation by spreading synthetic phospholipid mixtures containing both saturated and unsaturated phospholipids in a Langmuir trough and compressing to a surface pressure of 50 mN/m for deposition. Atomic force microscopy (AFM) was used to demonstrate the presence of multilayers through the topography of the deposited films. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used to map the location of each component in the mixture by imaging characteristic secondary ions. Our results indicate that multilayer structures can form in the presence of phospholipids only, however, specific unsaturated phospholipids are necessary. Our ToF-SIMS results demonstrate that unsaturated and saturated phospholipids distribute into multilayer and monolayer structures at similar concentrations - there is no evidence for phase separation between the monolayers and multi-layers.

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