indicating primarily elasticity changes, whereas amitriptyline and imipramine had equal effects on the different length gA, indicating primarily curvature changes.

2486-Pos

Thiazolidinediones Alter Lipid Bilayer Properties and Native Voltage-Gated Sodium Channel Function

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Thiazolidinediones (TZD) are selective peroxizome-proliferator receptor gamma (PPARγ) agonists that are used to treat hyperglycemia in type 2 diabetes. In addition to their hypoglycemic actions they have anti-inflammatory, anti-atherosclerotic and cardiovascular effects, but PPARγ activation does not account for all their actions. Three TZDs - troglitazone (Resulin), rosiglitazone (Avandia), and pioglitazone (Actos) - have been marketed; troglitazone was subsequently withdrawn due to hepatotoxicity and .a precursor TZD - ciglitazone- was discontinued after phase II trials. TZDs, with troglitazone being the most potent, modulate L-type calcium and delayed-rectifier potassium channels by a seemingly PPARγ-independent mechanism. This could result from the adsorption of the amphiphilic TZDs to the membrane/solution interface, which can alter bilayer properties such as thickness, intrinsic curvature and the elastic moduli, and thus membrane protein function. We therefore examined whether TZDs alter lipid bilayer properties. We exploited the sensitivity of gramicidin channels to changes in bilayer properties to test for TZD-induced bilayer effects. TZDs alter gramicidin channel function and shift the monomer-dimer equilibrium toward the conducting dimers. Using gramicidin channels of different lengths we find that the TZD effects do not vary with changes in hydrophobic mismatch. Increasing bilayer stiffness with cholesterol amplifies the TZD-mediated changes in gramicidin channel function. Based on the concentrations at which we observe changes in gramicidin lifetime and appearance frequency, the potency is troglitazone>rosiglitazone> ciglitazone>pioglitazone, consistent with their effects on native membrane proteins. We examined the TZDs effects in native membranes using neuronal voltage-gated sodium channels (Na_V) using whole-cell recordings. All TZDs caused a negative shift in the voltage-dependence of inactivation at concentrations similar to those that alter gramicidin channel function. Our results show that TZDs affect bulk membrane properties at concentrations that modulate native ion channels.

2487-Pos

NSAIDs Alter Bilayer Properties by a Common Mechanism

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Non-Steroidal Anti-inflammatory Drugs (NSAIDs) exert their primary action through inhibition of the Cyclo-oxygenase enzymes (COX-1 and COX-2). In addition to these COX-dependent effects, NSAIDs alter the function of a number of membrane proteins, by seemingly COX-independent mechanisms. Given that NSAIDs are amphiphiles, and that they modulate the function of different, structurally unrelated membrane proteins, we tested whether NSAIDs could alter lipid bilayer material properties at the same concentrations where they alter membrane protein function. To measure such changes in bilayer material properties, we used gramicidin A (gA) channels as molecular force transducers. We found that Aspirin, Salicylate, Sulindac, Sulindac Sulfide, Ibuprofen, Diclofenac and Flurbiprofen alter bilayer mechanical properties. At pH 7, NSAIDs increase both the lifetime and appearance rate of channels formed by both short (13-residue) and long (15-residue) gramicidin analogues, meaning that they shift the gA monomer<->dimer equilibrium toward the conducting dimers. The changes in gA channel function depend on the channel-bilayer hydrophobic mismatch, as we observe the larger effects on the shorter channels - the channels with the larger hydrophobic mismatch. We also found that, when comparing the effect of each NSAID, the relative changes in the lifetimes of the shorter and the longer channels could be described by the same linear relationship. We therefore conclude that NSAIDs decrease lipid bilayer stiffness by a common mechanism, through an increase in bilayer elasticity, and that specific channel-NSAID interactions are not involved. These effects were achieved at the high end of clinically relevant concentrations, and raise the possibility that in both the clinical and research setting, NSAIDs may have effects that arise from modulation of lipid bilayer mechanical properties.

2488-Pos

Supported Lipid Bilayers on Skeletonized Zirconium Phosphonate Surfaces For the Study of Transmembrane Proteins

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Supported lipid bilayers that can fully represent biological cell membranes are attractive biomimetic models for biophysical and biomedical applications. In previous work, we developed a new approach to engineer stable supported lipid membranes. This system uses the zirconium phosphonate substrate as a reactive surface that tethers the lipid membrane via a highly covalent bond between surface zirconium ions and divalent phosphate groups in the lipid assembly. An advantage of this approach is that the zirconium phosphonate can be applied to any surfaces (gold, glass, silicon) allowing different analytical techniques to be used on the same system. However, the covalent interaction between the zirconium phosphonate film and the inner lipid monolayer that is responsible for the bilayer stability restricts membrane components such as transmembrane proteins from penetrating into the membrane and retaining their mobility. In contrast to other approaches that incorporate pillars to support the bilayers, our strategy involves the creation of reservoirs beneath the supported lipid bilayers to accomodate transmembrane proteins. Skeletonized zirconium phosphonate surfaces have been designed using the Langmuir-Blodgett (LB) technique. Sizes of the nanometer-holes were varied by controlling the amount of octadecylphosphonic acid in mixed LB layers and characterized by atomic force microscopy. The skeletonization of the film was optimized so the hollow spaces were large enough to incorporate the proteins but small enough for the membrane to bridge the spaces. The efficiency of this system as a support for transmembrane proteins has been characterized by surface plasmon resonance enhanced ellipsometry (SPREE) using the proteins Annexin V and the maxi-K ion channel to demonstrate the utility of the system as a functional cell membrane. This concept is innovative in the area of bilayer platforms and can be used with membrane and transmembrane proteins.

2489-Pos

Critical Dependence of the Biophysical Activity of Pulmonary Surfactant Films on Temperature

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Pulmonary surfactant is a lipid-protein complex that stabilizes the respiratory surface of lungs. Once secreted into the alveolar spaces, surfactant adsorbs rapidly at the air-liquid interface reducing surface tension upon compression to near 0 mN/m. Within a given animal species, surfactant composition is influenced by development, disease, respiratory rate, and/or body temperature. In principle, surfactant collected from animals functions optimally at the body temperature 3of the animal at the time of sample collection.

Changes in temperature can alter the physical state and the molecular packing of surfactant membranes and films, potentially altering their biophysical performance. We have analyzed the effect of temperature on the structure of native surfactant, by differential scanning calorimetry (DSC) and fluorescence spectroscopy with the fluorescent probes DPH (Diphenylhexatriene) and Laurdan (6-Lauroyl-2-(N,N-dimethylamino)naphthalene). The spectral properties of these probes have been used to assess lipid packing and fluidity in surfactant as a function of temperature and compression state. The effect of temperature on the interfacial performance of surfactant has been evaluated by analyzing spreading capabilities in a surface balance and compression-expansion dynamics in a Captive Bubble Surfactometer. Native surfactant from porcine lungs showed optimal adsorption at temperatures around 37 oC, reaching minimal surface tensions below 2 mN/m upon quasi-static or dynamic compression-expansion cycling. Critical structural transitions at temperatures above 39oC led to reduced interfacial adsorption and impossibility of compressed films to reduce surface tensions below 20 mN/m, suggesting that surfactant composition has been optimized to work at a narrow interval of temperatures and that regulatory factors may be involved in adaptation of surfactant structures to changes in body temperature.

2490-Pos

The Effect of Membrane Spanning Peptides on Laurdan and Di-4-ANEPPDHQ Emission Spectra

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Laurdan and di-4-ANEPPDHQ are membrane order reporting probes whose peak emission wavelengths depend on the lipid environment. The probes report membrane order through different mechanisms, laurdan by sensing changes in the level of water penetration into the lipid bilayer and Di-4-ANEPPDHQ by sensing dipole potential changes in the membrane. Laurdan and di-4-ANEPPDHQ are excited by UV and blue light, respectively, and both show an ~50 nm blue shift in emission for membranes in liquid-ordered (l_o) phase versus membranes in liquid-disordered (l_d) phase.
Large unilamelar vesicles (LUVs) in l_o phase were created by mixing sphingomyelin, DOPC spiked with 5% DPPG and cholesterol in 1:1:2 ratio. LUVs in l_d phase were created only using DOPC spiked with 5% DPPG. Transmembrane polypeptides, mastoparan (a 14-residue peptide toxin isolated from wasp venom) or bovine prion protein (N-terminal residues 1-30), were added to 100 nm LUVs stained with 1μM laurdan or di-4-ANEPPDHQ in up to 1:10 protein/total lipid ratio. The laurdan and the di-4-ANEPPDHQ emission spectra were measured for both l_o and l_d phase LUVs before and after the addition of polypeptides and remained unchanged for all conditions. The integrity and size distribution of the LUVs upon addition of the polypeptides were determined by dynamic laser light scattering and no changes were detected. The insertion efficiency of the polypeptides into LUVs was determined by measuring their 3D polypeptide structure by circular dichroism. Both polypeptides had an alpha helical conformation compatible with them being inserted into the lipid bilayer. Our results suggest that the presence of proteins in biological membranes does not influence the spectra of laurdan and di-4-ANEPPDHQ showing that the dyes report solely on lipid order.

2491-Pos
Thermodynamics and Dynamics of the Formation of Spherical Lipid Vesicles
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We propose a free energy expression accounting for the formation of spherical vesicles from planar lipid membranes and derive a Fokker-Planck equation for the probability distribution describing the dynamics of vesicle formation. We found that formation may occur as an activated process for small membranes and as a transport process for sufficiently large membranes. We give explicit expressions for the transition rates and the characteristic time of vesicle formation in terms of the relevant physical parameters.

2492-Pos
Permeability of Model Stratum Corneum Lipid Membrane Measured using Quartz Crystal Microbalance: Non-Fickian Diffusion and Transient Membrane Swelling
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The stratum corneum (SC) is the outermost layer of the epidermis. Stacked intercellular lipid membranes found in the SC play a crucial role in regulating transport of water through the skin. In this work, we present a new method to determine the water permeability of a model SC lipid membrane using a quartz crystal microbalance (QCM) [*Langmuir*, **2009**, 25 (10), 5762-5766]. We investigate a model SC lipid membrane comprising an equimolar mixture of brain ceramide (CER), cholesterol (CHO) and palmitic acid (PA), and use QCM to determine the diffusivity (*D*), solubility (*S*) and permeability (*P*) of water vapor. We have found that the water transport in model skin lipid membranes can not be described in terms of *Fickian* process as the effective diffusion constant depends on the thickness of the lipid bilayer stacks. This may be due to slow equilibration process related to the membrane hydration. Using polarity-sensitive probe, PRODAN, we have found that the time scale of slow equilibration process is ~ 10 hrs, which may explain the non-Fickian behavior of a skin lipid membrane.

2493-Pos
Photophysical Studies of Novel Ruthenium Metal-Ligand Complexes Incorporated in Model Lipid-Membrane Bilayers
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We have designed and synthesized ruthenium metal-ligand complexes (MLCs) with amine- or acyl-reactive functional groups. The MLCs have potential as luminescent probes to investigate bio-macromolecular dynamics on the sub-microsecond-to-microsecond timescale. This timescale is of interest, for example, for analysis of the motions associated with macro-molecular assemblies and interactions of membrane-bound proteins. Here we report the photophysical and structural properties of MLCs (1) $[HRu(CO)(4,4'\text{-dicarboxybipyridyl})(PPh3)_2]^+$ $[PF_6]^-$ conjugated to dipalmitoyl-phosphatidyl-ethanolamine (DPPE), (2) $[HRu(CO)(bpy)(PPh_2C_2H_4COOH)_2]^+$ $[PF_6]^-$ conjugated to dimyristoyl-phosphatidyl-ethanolamine (DMPE) and (3) $[(CF_3CO_2)Ru(CO)(5\text{-aminophen})(Ph_2PC_2H_2PPh_2)]^+$ $[PF_6]^-$ conjugated to DPPE and cholesterol. These conjugates were incorporated in 100nm-diameter-unilamellar lipid-membrane vesicles to investigate the photophysical properties of the probes in a model membrane environment and to evaluate the utility of these probes for investigating the physical properties of lipid membranes. We are also investigating the photophysical behavior of MLCs in Nanodiscs, which are ~10nm-diameter phospholipid bilayers surrounded by a recombinant scaffold protein. We are using Nanodiscs as a platform for investigating the dynamics of transporter proteins, and we are using the MLCs as tools to characterize the physical properties of the Nanodisc-transporter assembly.

2494-Pos
Theoretical Design of Model Nanoparticles for Targeted Cell-Surface Binding
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Targeting therapeutic nanoparticles to circulating tumor cells is a crucial step in eradicating cancer from the body. The efficiency of this treatment relies on optimally binding surface ligands of the nanoparticles to the specific patterns of receptors uniquely displayed by the target cells prior to cellular uptake. We present a theoretical study that identifies the key characteristics of nanoparticles for binding to different types of afflicted cells, enabling enhanced detection and targeting capabilities.
Dynamic behavior of nanoparticle ligands and cell surface receptors are influenced by the properties of the surfaces on which they are attached. Thermal fluctuations enable the ligands and receptors to bind securely by probing varied conformations, but these systems are also subject to forces from elastic deformation of the surfaces and local tethering at receptor-ligand complexes. We introduce a novel simulation methodology that enables the coupling of particles' motion and the motion of their associated surface, which improves upon existing techniques that neglect the influence of particle dynamics on membrane motion. We explore how properties such as size, elasticity, and ligand mobility of a nanoparticle influence its ability to effectively associate with various target cells displaying unique receptor profiles. Our results elucidate the physical consequences of certain properties on receptor-ligand interactions, allowing systematic design of nanoparticles with improved abilities to bind specifically to a large array of tumor cells.

2495-Pos
PH.D Student
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Manipulations that alter the energetics of a chemical reaction often alter the activation free energy in linear correlation with the equilibrium free energy change. Such linear free energy relationship (LFERs) have been widely used to probe the energetics of transition states associated with protein folding and enzymatic catalysis. Nevertheless, the physical basis that underlie LFERs in such systems are not well understood, and it is not obvious how the slope of the linear relation should be interpreted. We show that the effects of amphiphiles on gramicidin A(gA) channel gating in lipid bilayers obeys a LFER and have studied the underlying mechanisms. The channel gating process provides a unique chemical reaction, in which structural changes in a model protein can be studied at the level of single molecules, while offering quantitative information about the energetics of a reaction transition state and its position on a spatial coordinate. We show that the LFER can be understood - and that the slope of the linear relation between changes in activation energy and equilibrium free energy for channel formation can be interpreted - by considering the effects of amphiphiles on the changes in bilayer elastic energy associated with channel gating. The use of amphiphile-induced changes in bilayer elastic properties provide a tool for studying LFERs associated with membrane protein function and folding.