

separation of the bound and free amphiphile using size exclusion chromatography and quantification of the bound amphiphile by HPLC. The high sensitivity of the method relies on an efficient increase in the concentration of the amphiphile by a minimum factor of 25 at the HPLC column, by injection of a very large volume coming from the size exclusion column and, for fluorescent amphiphiles, on the quantification in a solvent where it shows a very high fluorescent quantum yield.

The equilibrium partition is performed at the required temperature and the physical separation between both fractions of amphiphile is performed at low temperature to guaranty that the equilibrium is not displaced. The method was implemented for the fluorescent amphiphile NBD- C_{16} for which the desorption from POPC lipid bilayers is a very slow process ($k = 7.4 \times 10^{-5} \text{ s}^{-1}$ at 4°C (Cardoso, R., Master Thesis, Coimbra 2008)) conducing to less than 5% deviation from equilibrium during the 10 min required for separation of the two amphiphile fractions.

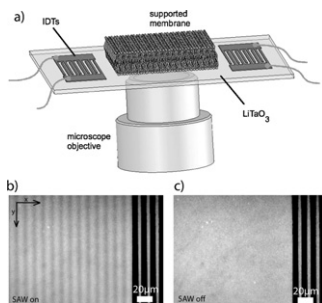
2482-Pos

Driven Dynamic Patterns of Supported Lipid Bilayers by Standing Surface Acoustic Waves

Martin Hennig^{1,2}, Jürgen Neumann^{3,2}, Achim Wixforth^{3,2}, Matthias Schneider^{4,2}, Joachim Rädler^{1,2}.

¹Ludwig-Maximilians-Universität, München, Germany, ²Center for NanoScience, München, Germany, ³Universität Augsburg, Augsburg, Germany, ⁴Boston University, Boston, MA, USA.

The in-plane structuring of lipid membranes not only provides new possibilities for the investigation of biomolecular processes in two dimensions, but also mimics a crucial property of the cell membrane. Here, we present a new tool, which allows to actively generate and control dynamic patterns in 2D supported lipid membranes by using nanoscopic standing surface acoustic shear waves (sSAWs) (a). The SAW couples to the membrane, inducing local accumulations of labelled lipids (b). After switching off the high frequency generator, the pattern decays with a diffusive timescale of seconds (c). Using a very thin piezoelectric substrate, sSAW driven manipulation of supported membranes is combined with high resolution fluorescence microscopy allowing to access the time evolution of driven domain formation, as well as the dynamics of single DNA molecules locally trapped in stripe-like domains on the surface of lipid membranes. Finally, the tool presented does not only extend the concept of supported lipid membranes in basic research, but also offers a variety of practical applications like particle filters over a wide range in size, controlled formation of dynamic cell patterns or single molecule transport with protein separation.

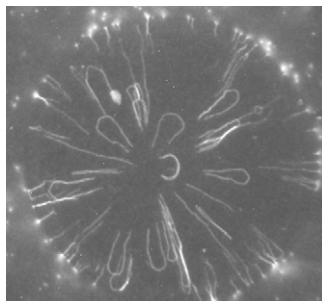


2483-Pos

Double End-Grafted DNA as Force Sensors for Bio-Adhesion Spreading Yuting Sun.

ICS, Strasbourg, France.

Cell-adhesion events involve often the formation of a contact region between phospholipid membranes decorated with a variety of bio-macromolecular species. We study the spreading of a bio functional phospholipid bilayer on a carpet of double end-grafted DNAs. The spreading process scrapes and staples the chains between the membrane and the substrate. The final stapled DNA shape is function of both the internal chain tension and the forces applied by the bilayer. We show that by using the well known force extension relationship for a DNA molecule we can reveal the forces at play during the formation of the adhesion patch.



2484-Pos

High Throughput Gramicidin-Based Fluorescence Assay to Screen for Small Molecules' Bilayer-Perturbing Potential

Helgi I. Ingolfsson^{1,2}, Olaf S. Andersen¹.

¹Weill Cornell Medical College, New York, NY, USA, ²Tri-Institutional Training Program in Computational Biology and Medicine, New York, NY, USA.

Many small molecules used to modulate biological function are amphiphiles that adsorb at the bilayer/solution interface and thereby alter lipid bilayer properties. Such changes in bilayer properties can lead to altered membrane protein function due to the hydrophobic coupling between the host bilayer and its embedded proteins. Amphiphile-induced changes in lipid bilayer properties may, therefore, provide a mechanistic basis for the "off target" effects of drugs and other biologically active molecules. We have previously developed electrophysiological assays for changes in lipid bilayer properties, as sensed by bilayer-spanning proteins, using the channels formed by the linear gramicidins as probes. Gramicidin channels are mini-proteins formed by the transbilayer dimerization of two non-conducting subunits; they are sensitive to changes in their membrane environment, which renders them excellent probes for monitoring changes in bilayer properties. We now report a fluorescence assay for detecting changes in bilayer properties, using the linear gramicidins as probes. The assay is based on measuring the time course of fluorescence quenching from fluorophore-loaded large unilamellar vesicles, due to the entry of a quencher through the gramicidin channels. The fluorescence method presented is scalable and suitable for both mechanistic studies and high-throughput screening of small molecules for bilayer-perturbing, and potential off-target, effect. To illustrate the validity and power of this approach, we have tested compounds with bilayer-modifying effects that previously have been characterized using the electrophysiological (single-channel) gramicidin approach. We find that the methods are in good agreement. We also have undertaken a systematic study of the bilayer-perturbing effect of short- and intermediate-chain length alcohols (methanol through *n*-octanol, isopropanol, 2-butanol and tert-butanol) as well the fluorinated alcohols (trifluoroethyl alcohol, hexafluoroisopropanol and nonafluoro-tert-butyl alcohol). These compounds alter lipid bilayers properties at the concentrations at which they alter membrane protein function.

2485-Pos

Antidepressants Modify Lipid Bilayer Properties

Ruchi Kapoor¹, Helgi I. Ingolfsson¹, Roger E. Koeppe II², Olaf S. Andersen¹.

¹Weill Cornell Medical College, New York, NY, USA, ²University of Arkansas, Fayetteville, AR, USA.

Antidepressants are the most commonly prescribed drugs in the U.S. The two major classes of antidepressants - tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) - alter serotonin and norepinephrine availability in the brain, though it remains unclear whether all of their antidepressive effects can be ascribed to changes in the serotonergic system. In addition to their main clinical use, these compounds also have off-label uses for conditions such as premature ejaculation and migraines. The mechanism(s) underlying these latter effects are unknown, but TCAs and SSRIs alter the function of many proteins, including voltage- and ligand-gated channels. Membrane proteins span the lipid bilayer, and are coupled to the bilayer through hydrophobic interactions, such that conformational changes underlying their function may involve local reorganization of the surrounding lipids. Such bilayer deformations incur energetic costs that vary with bilayer properties. Since the adsorption of amphiphiles alters bilayer properties, they may also alter the bilayer contribution to the free energy difference between protein conformations. We examined whether the lipid bilayer could mediate the non-serotonergic effects of the TCAs, amitriptyline and imipramine, and of the two enantiomers of the SSRI fluoxetine. Gramicidin A (gA) channels were used as probes for changes in bilayer properties in three different implementations: bilayer-punch, tip-dip, and a fluorescence assay. Both TCAs and SSRIs increased gA channel activity in a dose-dependant manner irrespective of hydrocarbon presence, indicating that they increase bilayer elasticity. In all three systems, fluoxetine is a more potent bilayer modifier than the TCAs with no enantiomer-specific differences. Single-channel experiments (bilayer-punch and tip-dip) show that the antidepressants increase channel lifetime and appearance rate. The fluoxetines have a larger effect on shorter channels,

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

Radda Rusinova¹, Karl F. Herold¹, Roger E. Koeppe II², Hugh C. Hemmings, Jr.¹, Olaf S. Andersen¹.

¹Weill Cornell Medical College, New York, NY, USA, ²University of Arkansas, Fayetteville, AR, USA.

Thiazolidinediones (TZD) are selective peroxisome-proliferator receptor gamma (PPAR γ) 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 PPAR γ 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 PPAR γ -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 ν) 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

Subhi J. Al'Aref^{1,2}, Roger E. Koeppe II³, Olaf S. Andersen².

¹New York Presbyterian Hospital, New York City, NY, USA, ²Weill Cornell Medical College, New York City, NY, USA, ³University of Arkansas, Fayetteville, AR, USA.

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 \rightleftharpoons 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

Roxane M. Fabre, George O. Okeyo, Daniel R. Talham.

University of Florida, Gainesville, FL, USA.

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 accommodate 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

M. Victoria Picardi, Jesús Pérez Gil.

Universidad Complutense Madrid, Madrid, Spain.

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 30f 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 °C, reaching minimal surface tensions below 2 mN/m upon quasi-static or dynamic compression-expansion cycling. Critical structural transitions at temperatures above 39°C 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

Jelena Dinic¹, Henrik Biverstahl², Lena Mäler², Ingela Parmryd¹.

¹Wenner-Gren Institute, Stockholm University, Stockholm, Sweden,

²Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.