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 ($k_{-}=7.4\times10^{-5}$ 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
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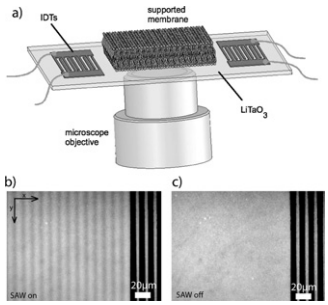
The in-plane structuring of lipid membranes not only provides new possibilities for the investigation of biomolecular processes in two dimensions, but also mimics a crucial property of the cell membrane. Here, we present a new tool, which allows to actively generate and control dynamic patterns in 2D 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
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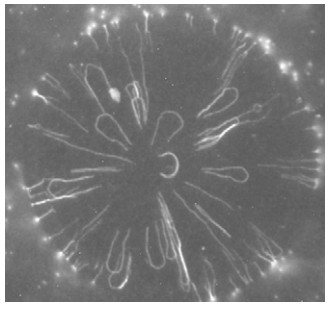
Cell-adhesion events involve often the formation of a contact region between phospholipid membranes decorated with a variety of bio-macromolecular species. We study the spreading of a bio functional phospholipid bilayer on a carpet of double end-grafted DNAs. The spreading process scrapes and staples 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
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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 alcoho, 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
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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 enantionmers 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,