Membrane Physical Chemistry III

2477-Pos

Lipid Mediated Interaction of Transmembrane Helices as Studied by a Mesoscopic Model

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The role transmembrane helices (TMH) play in biological systems includes creating ion transport pathways, cell signaling and facilitating photon absorption in photosynthetic complexes. The packing and spatial organization of those helices was found to be important for their functional properties. It was shown (Walters & DeGrado, PNAS (2006); 13, 37) that despite the large number of available conformations, experimentally observed helix-helix interactions can be classified into very few interaction clusters. This suggests that a basic, universal set of interactions might govern the helix packing. Using a coarse-grained model we investigate the interaction of helical peptides in a lipid bilayer using the dissipative particle dynamics (DPD) simulation technique. We incorporate in our model basic hydrophobic-hydrophilic interactions without referencing a specific TMH, thereby studying the common motifs in lipid mediated protein interactions. Our model successfully reproduces the effect of hydrophobic mismatch on peptides in a lipid bilayer (de Meyer, Venturoli & Smit, Biophys. J (2008); 95, 4) and predicts a selective aggregation pattern. A more detailed representation of a helix further reveals the characteristics of the helix-helix interactions

2478-Pos

The Interaction of Curcumin with Phospholipid Model Membranes. a Study using Differential Scanning Calorimetry, NMR, X-Ray Diffraction and Infrared Spectroscopy

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Curcumin is a polyphenol present in turmeric, widely used in Asian traditional medicine and cooking, which has many and diverse biological effects and is found incorporated in membranes. We have studied the mode in which curcumin modulates the physical properties of 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) multilamellar membranes and 1, 2-dielaidoyl-sn-glycero-3-phosphoetnanolamine (DEPE). Curcumin disordered DPPC membranes at temperatures below Tc as seen through DSC, FT-IR, 2H-NMR, WAXD and SAXD. The decrease induced in Tc, suggests that curcumin is oriented in the bilayer with its main axis parallel to the acyl chains. Above Tc curcumin also introduced disordering as seen by FT-IR. FT-IR also showed that curcumin alters the conformation of the polar group of DPPC, increasing the percentage of unhydrated C=O groups, but however it does not form hydrogen bonds with neither the C=O group nor the phosphate group of DPPC. SAXD showed a remarkable increase in the repeating spacings by the presence of curcumin probably indicating the formation of a ripple phase. A partial phase diagram was built, which suggest the formation of a phospholipid/curcumin complex given place to immiscibilities in both the fluid and the rigid states, between curcumin and DPPC. Additionally DEPE was used to test the effect of curcumin on its polymorphism, and it was found that the temperature at which HII phase is formed was decreased, indicating that curcumin favours negative curvature of the membrane, which may be important to explain its effect on membrane dynamics and on membrane proteins or on proteins which may be activated through membrane insertion.

2479-Pos

Assesment of the Biophysical Parameters of Platelet Membrane in Leukemic Patients

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The clinical history of myeloproliferative and myelodysplastic disorders is often complicated by thromboembolic or hemorrhagic events. The mechanisms of these major and life threatening complications remain unclear.
Membrane organization influences many of the unique cellular functions and is strongly correlated (among other factors) to the membrane lipid composition; it may be evaluated by following up the membrane fluidity and aggregation properties of the cell. In our work, we try to correlate changes in platelets membrane fluidity and aggregation parameters with the clinical status of the patient disease.
Membrane fluidity and aggregation properties of platelets collected from 176 patients suffering of various entities of myeloid malignancies as well as from 34 healthy controls were monitored along one to 6 month in the attempt to establish a correlation between membrane organization changes and alterations of the platelet function which accompany the disease.
Membrane fluidity was assessed by fluorescence anisotropy measurements. The platelet membrane shows to be more rigid compared with controls/normal regardless of the clinical type of myeloproliferative disorder. However patients with severe clinical status due to acute myeloid leukemia have a more fluid membrane compared to the same patients found previously in a better state.
Aggregation was assessed by optical methods (Chronolog Aggregometer). The lag phase amplitude and duration, the slope, amplitude and secretion phase of the aggregation curve were monitored revealing that the leukemic platelet response is reduced for all agonist reagents (ADP, epinephrine, collagen and risocetin). The reduction of the epinephrine response is more pronounced comparatively to the response to other reagents. These results suggest the possible mechanisms of platelets disorders induced by the disease.

2480-Pos

Characterization of a Homologous Series of Fluorescent Fatty Amines. Photophysics, Aqueous Solubility and Binding to Albumin and to POPC Bilayers

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The *7-nitrobenz-2-oxa-1,3-diazol-4-yl (*NBD) is a small and very polar group whose fluorescence quantum yield strongly depends on the polarity of its environment. When bound to the polar headgroup of lipid molecules it allows the characterization of the structure and dynamics of lipid bilayers. The solvent dependence of its fluorescence has been exploited by this research group to characterize the kinetics and thermodynamics of the interaction of different amphiphiles with lipid bilayers and proteins.
In this work we report on the synthesis and characterization of a homologous series of fluorescent fatty amines (NBD-Cn; n=4, 6, 8, 10, 12, 14 and 16). At 25°C and pH=7.4, the critical aggregation concentration (CAC) in aqueous media range from $2x10^{-4}$ M for NBD-C_4 to $4x10^{-9}$ M for NBD-C_{12}. The partition coefficient to lipid bilayers prepared from *1-palmitoyl-2-oleoyl phosphatidylcholine* (POPC) was also measured for the amphiphiles with a CAC>20 nM (NBD-C_4 to NBD-C_{10}) and ranged from $9.5x10^2$ to $3.6x10^5$, with a $\Delta\Delta G$=-4.9 ± 0.5 kJ/mol *per* ethyl group. The amphiphiles interacted efficiently with Bovine Serum Albumin (K_B=$1.7x10^4$ and $7.9x10^6$ M^{-1} for NBD-C_4 and NBD-C_{10} respectively) and this was inhibited by fatty acids indicating that binding occurs essentially in the same binding site.
Some photophysical properties of the amphiphiles in POPC bilayers were also measured and we found no significant variation along the series indicating that the NBD group is located in a region with the same properties regardless of the length of the non-polar group. An exception was noted for the case of NBD-C_{14} that showed somewhat smaller fluorescence anisotropy. The amphiphiles lifetime decay observed was mono-exponential in water or methanol but when inserted in POPC bilayers a bi-exponential law was required.

2481-Pos

New Method for the Measurement of Binding Constants for Amphiphiles with a Very Small Solubility in Aqueous Media

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The aqueous solubility of the monomeric form of most amphiphiles is relatively small and above a certain concentration, the critical aggregation concentration (CAC), they tend to form aggregates where the contact between their non-polar moieties and water is minimized. The affinity of the amphiphiles to hydrophobic environments, such as proteins or lipid bilayers, may be obtained by equilibrium titration with the binding agent but its correct evaluation requires the use of amphiphile concentrations below their CAC which, at times, can be extremely low.
In this work we develop a method where the partition of amphiphiles between water and lipid bilayers may be accurately measured for amphiphiles with a CAC in the sub-nanomolar range. The method is based on the physical

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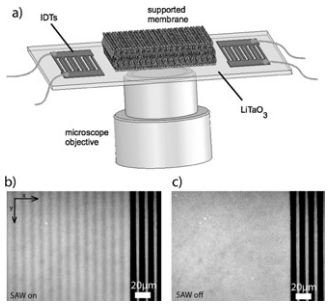
The in-plane structuring of lipid membranes not only provides new possibilities for the investigation of biomolecular processes in two dimensions, but also mimics a crucial property of the cell membrane. Here, we present a new tool, which allows to actively generate and control dynamic patterns in 2D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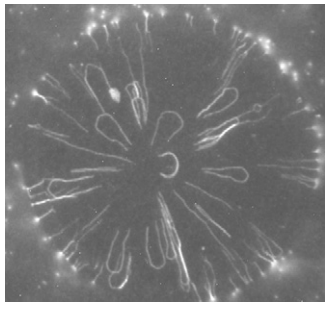
Cell-adhesion events involve often the formation of a contact region between phospholipid membranes decorated with a variety of bio-macromolecular species. We study the spreading of a bio functional phospholipid bilayer on a carpet of double end-grafted DNAs. The spreading process scrapes and staples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