Platform BC: Membrane Physical Chemistry II

3253-Plat

Interaction of DNA-PAMAM Dendrimers with a Model Biological Membrane

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The systemic delivery of DNA for gene therapy requires control of DNA compaction by an agent, such a lipid, surfactant or a polymer (e.g. cationic dendrimers). The crucial step here is to make the DNA cross the membrane to gain entry into the cell. Poly (amido amine) (PAMAM) dendrimers show great promise as synthetic gene-transfection agent. We have studied the structure of the complexes formed between DNA and PAMAM dendrimers as well as their interaction with model membranes, using a range of biophysical experimental techniques and simulation. We noted that the structure of the complex formed strongly depends on the generation of the dendrimer. The results show that generation 2 (G2) and 4 (G4) PAMAM dendrimers are able to penetrate surface deposited bilayers, consisting of palmitoyl oleoyl phosphatidyl choline as shown by neutron reflectometry (NR). The ability of the dendrimers to penetrate lipid bilayers is confirmed by coarse-grained simulations. The experimental and simulation data show that the DNA-dendrimer complex has a reduced ability to penetrate the bilayer, compared to the naked dendrimers. We will discuss these results in relation to the design of efficient transfection mediators with membrane permeating ability.

3254-Plat

Solid State NMR Studies on Acylated Transmembrane Peptides Driving Membrane Fusion

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The fusion of biological membranes is mediated by integral membrane proteins with α-helical transmembrane segments (TMSs). Additionally, those proteins are often modified by the covalent attachment of hydrocarbon chains. Previously, a series of *de novo* designed α-helical peptides with mixed Leu/Val sequences was presented, mimicking fusogenic TMSs in model membranes (Hofmann et al., Proc. Natl. Acad. Sci. USA 101 (2004) 14776-14781). From this series, we have investigated the peptide LV16 (KKKWLVLVLVLV LVLVLVLVKKK), which was synthesized presenting either a free N-terminus or an N-acylation of 2, 8, 12, or 16 carbons. We used ^{2}H and ^{31}P NMR spectroscopy to investigate the structure and dynamics of those peptide lipid modifications in POPC and DLPC bilayers and compared them to the hydrocarbon chains of the surrounding membrane. Except for the C-2 chain, all peptide acyl chains were found to insert well into the membrane. This can be understood from the high local lipid concentration, which the N-terminal lipid chains experience. The insertion of these peptides did not influence the membrane structure and dynamics as seen from ^{2}H and ^{31}P NMR. In spite of the fact that the longer acyl chains insert into the membrane, there is no perfect length adaptation. Even the C-16 chain on the peptide, which could match the length of the POPC palmitoyl chain exhibited lower order parameters in the upper chain, which get closer and finally reach similar values in the lower chain region. ^{2}H NMR square law plots reveal a slightly more dynamic characteristic of the peptide acyl chains compared to the surrounding phospholipids. In spite of the significantly different chain lengths of the acyl chains, the fraction of *gauche* defects in the inserted chains is constant, suggesting similar entropies of the inserted chains.

3255-Plat

Effect of PEG-based Biocompatible Polymers on the Response of Lipid Vesicles under External Stimuli

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Cell membrane dysfunction due to loss of structure integrity is the pathology of tissue death in trauma, muscular dystrophies, reperfusion injuries and common diseases. It is now established that certain PEG-based biocompatible polymers, such as Poloxamer 188, Poloxamine 1107 and PEG, are effective in sealing of injured cell membranes, thus can prevent acute necrosis if delivered timely after injury. Despite these broad applications of PEG-based polymers for human health, the fundamental mechanisms how PEG-based polymers interact with cell membranes are still under debate. Here, the effects of PEG-based biocompatible polymers on phospholipid membrane integrity under external stimuli (osmotic stress and oxidative stress) were explored using model cell membranes - giant unilamellar vesicles. Through fluorescence leakage assays and time-lapse fluorescence microscopy, we directly monitored the loss of structural integrity of single fluorescent dye-loaded GUVs under different stimuli, and observed the effects of triblock copolymers on these damaged membranes. We find that the interaction of the polymers with the lipid membrane involves two stages: an adsorption (I) and an insertion (II) state. We propose that the adsorption of the polymers on the membrane surface is responsible for the cell membrane resealing process due to its corralling effect, which is evidenced by slow-down of surface hydration dynamics upon adsorption of the polymers. In the insertion state, on the other hand, the polymers disturb the packing of phospholipids due to the mismatch in size and hydrophobicity of the PPO block with the lipid hydrocarbon tails, increasing the membrane permeability. Our results indicate that the biomedical application of PEG-based polymers, either as cell membrane resealing agents or as accelerators for drug delivery, is directed by the delicate balance between the adsorption and insertion of the polymers to the cell membranes.

3256-Plat

Detergent Effects on Membranes at Sub-Solubilizing Concentrations: Transmembrane Lipid Motion, Bilayer Permeabilization and Vesicle Lysis/reassembly are Independent Phenomena

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Soluble amphiphiles, or detergents, are known to produce a number of structural and dynamic effects on membranes, even at concentrations below those causing membrane solubilization, i.e. at the so-called stage I of detergent-membrane interaction. The main sub-solubilizing detergent effects on membranes are: transmembrane lipid motion (flip-flop), breakdown of the membrane permeability barrier leakage, and vesicle lysis / reassembly.

For a proper understanding of membrane solubilization by detergents it is important to assess whether the various effects seen at sub-solubilizing surfactant concentrations occur independently from each other, or else they are interconnected by cause-effect relationships, so that they can be interpreted as necessary steps in the overall process of solubilization. In order to answer this question we have explored the three above-mentioned effects, i.e. flip-flop, leakage and lysis / reassembly, apart from solubilization, in model (large unilamellar vesicles) and cell (erythrocyte) membranes. Five structurally different surfactants, namely chlorpromazine, imipramine, Triton X-100, sodium dodecylsulfate and sodium deoxycholate have been used. Each of them behaves in a unique way. Our results reveal that lipid flip-flop, vesicle leakage and vesicle lysis/reassembly are independent phenomena between them and with respect to bilayer solubilization, so that they can not be considered as necessary stages of a higher-order unified process of membrane solubilization by detergents.

3257-Plat

Effect of Cations on the Nanomechanical Response of Phospholipid Model Membranes. A Force Spectroscopy Study

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How do metal cations affect the stability and structure of phospholipid bilayers? Which role does ion binding play in the insertion of proteins and in the overall mechanical stability of biological membranes? To characterize such effects, several theoretical and microscopic approaches have been proposed in the past to study the mechanical properties of lipid bilayers. While providing crucial information, molecular dynamics simulations can not completely deal with the extraordinary complexity of biological membranes. Experimental techniques also have problems when it comes to test ion binding to lipid bilayers in an accurate way. Hence, a new perspective from the nanometric scale [1,2], where most of the specific molecular phenomena are sensitive, was introduced being Atomic Force Spectroscopy an essential tool to examine the lipid bilayers structure and behaviour. So, we used Force Spectroscopy to quantitatively characterize the nanomechanical resistance as a function of the electrolyte concentration and composition thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve. By systematically testing two model membranes, DPPC and DPPE, immersed in an electrolyte containing a series of either one monovalent (Li^{+} to Cs^{+}) or divalent cation (Mg^{2+} and Ca^{2+}) we provide a wealth of information which unambiguously proves an independent contribution of each ion to the gross mechanical resistance, reporting quantitative measurements for the membrane elastic modulus and also for its

plastic properties. So, this work deals with the need of assessing the effect of different cations in the structure of phospholipid membranes.
[1] Effect of ion-binding and chemical phospholipid structure on the nanomechanics of lipid bilayers studied by force spectroscopy, Biophys J 89 (2005) 1812-1826
[2] Nanomechanics of lipid bilayers:heads or tails?, under review (2009)

3258-Plat
Action of an Antiparasitic Peptide Active against African Sleeping Sickness in Biomembrane Models
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Peptides with trypanocidal activity are promising compounds for the treatment of African Sleeping Sickness, which have motivated the research into the ability of these compounds to disrupt the protozoan membrane. In this present study, we used the Langmuir monolayer technique to investigate the surface properties of an antiparasitic and zwitterionic peptide, namely S-(2,4-dinitrophenyl) glutathione di-2-propyl ester, and its interaction with a model membrane comprising a phospholipid monolayer, dipalmitoyl phosphatidyl choline (DPPC). The peptide formed a stable Langmuir monolayer, whose main feature of its surface pressure-area isotherm was the presence of a phase transition accompanied by a negative surface compressional modulus, which was attributed to the aggregation upon compression due to intermolecular bond associations of the molecules. This was inferred from surface pressure and surface potential isotherms, Brewster angle microscopy (BAM) images, Polarization modulation-infrared reflection-adsorption spectroscopy (PM-IRRAS), and dynamic elasticity measurements by the pendant drop technique. When co-spread with dipalmitoyl phosphatidyl choline (DPPC), the drug affected both the surface pressure and the monolayer morphology, even at high surface pressures and with low amounts of the drug. The results were interpreted by assuming a repulsive, cooperative interaction between the drug and DPPC molecules. Such repulsive interaction and the large changes in fluidity arising from drug aggregation may be related to the disruption of the membrane, which is key for the parasite killing property.

3259-Plat
Quantifying Interactions between Nanoparticles and Model Cell Membranes
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Due to their small size, nanoparticles (NPs) have the ability to penetrate pulmonary and vascular tissue, and as a result, are classified as potential human carcinogens. To examine factors that influence the interaction of functionalized NPs with cells in the body, the outer leaflet of the cell membrane was modeled with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipid monolayers. Polystyrene NPs without chemical modification and those functionalized with negatively charged carboxylic acid or positively charged amine groups, all with 60nm diameters, were introduced to the monolayer while environmental effects of pH and ionic strength were systematically altered. NPs displayed the largest interaction with the film in the presence of ions. At bilayer equivalent pressure, the aminated and carboxylated NPs showed appreciable monolayer insertion (with approximate area increases of 14% and 4.5%, respectively), whereas plain NPs solubilized the phospholipid, removing it from the air/water interface. All of these NP solutions contained a small mol% of detergent to prevent aggregation. Aminated and carboxylated polystyrene NPs free from additional surfactant were used to determine the effect detergent had on the surface activity of the NPs. Results will also be shown from experiments designed to determine the effect of NP charge and size (120nm), as well as how different lipid systems changed the fundamental interaction.

3260-Plat
A Water Gradient can be used to Regulate Drug Transport across Skin - A Responding Membrane
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At normal conditions there is a substantial water gradient over the skin as it separates the water-rich inside of the body from the dry outside. This leads to a variation in the degree of hydration along the skin and changes in this gradient may affect the structure and function of skin. In this study we raise the question: How do changes in the water gradient across skin affect its permeability? We approach this problem in experiments that permit strict control of the gradient in the chemical potential of water. The results demonstrate that an external water gradient can be used to regulate transport of drugs across the skin. It is shown that the permeability of the skin barrier increases abruptly at low water gradients, corresponding to high degrees of skin hydration, and that this effect is reversible. This phenomenon is highly relevant to drug delivery applications due to its potential of temporarily opening the skin barrier for transdermal delivery of drugs and subsequently closing the barrier after treatment.
The results are explained on basis that the skin is a responding membrane, for which small changes in the environment can lead to major changes in membrane structure, which in turn affect its transport properties. We have in parallel theoretical modeling and experimental studies in model systems shown how a water gradient across multilayer lipid membrane can be used as a regulating mechanism to control the barrier properties. These principles are here applied to the barrier of stratum corneum, the upper layer of the human skin, where it can provide an explanation for the experimental findings that a water gradient can be used to regulate drug transport across the skin.

Platform BD: Membrane Transporters & Exchangers

3261-Plat
Coevolving Amino Acid Positions in Exporter-Type ABC Proteins
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Residue-residue interactions define the protein fold, and their dynamic interplay mediates conformational rearrangements between global states, such as the outward- or inward-facing conformations of transporters. These physical interactions constrain sequence evolution by coupling the pattern of amino acid substitution at interacting positions (coevolution). Thus, identification of coevolving positions can provide structural and mechanistic insights at the resolution of single residues.
Here we identified coevolving positions in the OAD and DPL families of the ABC superfamily. These families harbor exporters involved in multidrug resistance like MDR1/Pgp (DPL) and MRPs (OAD), as well as the CFTR chloride channel (OAD) linked to cystic fibrosis. We generated multiple sequence alignments separately for OADs and DPLs, and analyzed them with three different statistical methods.
The three methods yielded somewhat different results likely due to their limited accuracy and differences in their assumptions about mechanisms of coevolution. Nonetheless, the results are validated by three lines of structural evidence, all supporting the hypothesis that direct physical interactions play a major role in coevolution. First, coevolution statistics were significantly linked to spatial distance in a 3D structural model. Second, the methods agreed better if only contacting positions were considered. Third, coevolving pairs were separated in sequences according to the periodicity of alpha helices and beta sheets. We present sets of coevolving pairs that link different transmembrane helices, or that link the coupling helices to the ATP-binding cassettes. Our findings provide specific, testable hypotheses for mutational and crosslinking studies on the detailed transport mechanisms of clinically relevant ABC proteins such as those underlying cystic fibrosis and multidrug resistance.
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3262-Plat
The Origin of Nucleotide Dependence of Conformational Changes in ABC Transporters
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ATP-binding cassette (ABC) transporters are one of the most ubiquitous membrane transporters. They are all powered by ATP binding and hydrolysis in the two highly conserved cytoplasmic nucleotide binding domains (NBDs). It has been structurally established that the NBDs adopt a closed dimeric conformation only in the ATP-bound state, while appearing as open dimers or separate monomers in their nucleotide-free and ADP-bound states. The origin of such conformational changes, however, is yet to be characterized. To study the mechanism of nucleotide-dependent conformational changes, an extensive set of molecular dynamics simulations was performed on several intact ABC transporter structures and in various nucleotide binding states. Through these simulations we identify significantly large electrostatic potential regions centered at each subdomain of the NBDs in all ABC transporters simulated. Interestingly, the