

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.

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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.

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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 var-

iation 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

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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.

[Supported by DK 51767]

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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