plastic properties. So, this work deals with the need of assessing the effect of different cations in the structure of phospholipid membranes.
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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

magnitude and location of the charge centers are independent of the nucleotide binding state of the NBDs. We propose that the repulsion between these charge centers is the main drive for the large separation between the NBDs in the absence of ATP. In particular, a conserved charged residue in the helical subdomain of the NBD is found to significantly contribute to the electrostatic repulsion between the NBD monomers. Removing the charge of this conserved residue during the MD simulations results in drastic changes of the NBD conformations, such that the NBDs are unable to complete their opening or closing motion in response to the bound nucleotide, hence a semi-open conformation is maintained in the mutant NBDs both in nucleotide-free and ATP-bound states.

3263-Plat

Functional Rotation of the Transporter AcrB: Insights into Drug Extrusion from Simulations

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The tripartite complex AcrAB-TolC is the major efflux system in Escherichia coli. It extrudes out of the bacterium a wide spectrum of noxious compounds, including many novel antibiotics. Its active part, the homotrimeric transporter AcrB, is responsible for the selective binding of substrates and energy transduction. Based on the available crystal structures and biochemical data, the transport of substrates by AcrB has been proposed to take place via a functional rotation, in which each monomer neatly assumes a particular conformation. However, there is no molecular-level description of the conformational changes associated with such a rotation and of their connection to drug extrusion. To obtain insights thereon, we have performed extensive targeted molecular dynamics simulations mimicking the functional rotation of AcrB containing the antibiotic doxorubicin, one of the two substrates that were co-crystallized so far. The simulations, including almost half a million atoms, have been used to test several hypotheses concerning the structure-dynamics-function relationship of this transporter. Our results indicate that, upon induction of conformational changes, the substrate detaches from the binding pocket and approaches the gate to the central funnel. Furthermore, we provide strong evidence for the proposed peristaltic transport involving a zipper-like closure of the binding pocket, responsible for the displacement of the drug. A concerted opening of the channel between the binding pocket and the gate further favors the displacement of the drug. This microscopically well-funded information allows to identify the role of specific amino acids during the transitions and to shed light on the functioning of AcrB.

3264-Plat

Protein Dynamics in the Transport Cycle: NMR Study of the Multidrug Resistance Transporter, EmrE

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Bacterial antibiotic resistance is a growing public health concern. One mechanism of resistance arises through drug export by multidrug resistance transporters. To fully understand the function of these proteins requires multiple structures plus kinetic and thermodynamic data to characterize the transport cycle. NMR offers a unique tool to obtain all of this information. The small size of the small multidrug resistance transporter, EmrE, makes it ideal for such studies. EmrE is a secondary active transporter in *E. coli* that harnesses the H+ gradient to export a broad range of polyaromatic cations from the cell, thus conferring resistance to drugs of this type. Protein conformational change is required for proper transport, allowing alternating access to either side of the membrane in response to substrate binding. We have solubilized EmrE in isotropic bicelles and have found that two conformations are present under these conditions. These two states are interconverting slowly on the NMR timescale, allowing us to study this transporter in action with atomic resolution.

3265-Plat

Molecular Basis of the "Alternate Access Model" in the Cation-Substrate Symporter Mhp1 from Computer Simulations and X-Ray Crystallography

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A common structural motif is emerging for a wide class of substrate-cation symporters. The fold of the amino acid-sodium symporter LeuT is shared by proteins unrelated by sequence identity such as the galactose-sodium symporter vSGLT, the nucleobase-cation symporter Mhp1, the betaine-transporting osmoregulator BetP, and amino acid-proton transporters AdiC, and ApcT. The "alternating access" model explains transport as cycling between at least three distinct conformational states that connect a central binding site to either the extracellular or the intracellular compartment. The crystal structures solved so far can be broadly categorized in these three conformations, outward facing (LeuT, Mhp1, BetP, AdiC), occluded (Mhp1, BetP, ApcT), and inward facing (vSGLT). We are currently studying the crystal structure of Mhp1 hydantoin transporter from Microbacterium liquefaciens in the inward facing open state. Together with the previous structures [1] a full picture of the conformational change occurring during transport emerges. Dynamic importance (DIMS) molecular dynamics (MD) simulations allow us to connect these three states with continuous transition trajectories. The combination of structural and simulation data puts the alternate access model on a firm structural basis and will facilitate future detailed studies of the energetics of cation-substrate coupled transport.

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[1] Weyand et al. (2008) Science 322, 709-713.

3266-Plat

Symmetry in the Structure of the Glutamate Transporter GltPh Suggests Conformation of an Alternate State

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Glutamate transporters regulate synaptic concentrations of L-glutamate to prevent excitotoxicity in nerve cells. Current crystal structures of GltPh, an archeal homologue of the Glutamate transporters, have an extracellular-facing binding site. The alternating access theory implies that a cytoplasm-facing state also exists. In order to model this state, we have identified two distinct sets of inverted-topology repeats, and used these repeats to model an inward-facing conformation of the protein. Specifically, we modeled the sequence of each repeat on the structure of its partner. In this model, a portion of the protein containing two transmembrane helices (TM7 and 8) and two helical hairpins (HP1 and HP2) is displaced relative to the crystal structure so that the binding site is exposed to the cytoplasm. In order to validate our model, pairs of cysteines were introduced into the neuronal glutamate transporter EAAC1 at positions that were greater than 27 Ångstroms apart in the outward-facing crystal structure, but closer to 10 Ångstroms apart in our model. Transport in these mutants was activated by pretreatment with the reducing agent dithithreitol. Once treated with the oxidizing agent copper(II)(1,10-phenantroline)3, however, activity ceased. Importantly, this inhibition was potentiated under conditions expected to promote the inward-facing conformation. This suggests that during the transport cycle these cysteines come within the range necessary to crosslink, as predicted by our inverted-topology repeat model of the cytoplasm-facing state. Previously, an alternative conformational state of the LeuT transporter was also modeled using inverted-topology repeats, suggesting that inverted-topology repeats may provide a general and elegant solution to the requirement for two symmetry-related states in a single protein.

3267-Plat

Opposite Movements of the External Gate in Glutamate Transporters upon Binding Different Cotransported Ligands Measured by EPR

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Hairpin two (HP2) has been proposed as the extracellular gate of glutamate transporters. To test this hypothesis, we use double site-directed spin-labeling electron paramagnetic resonance spectroscopy on the bacterial transporter Glt_{Ph} to examine conformational changes in HP2. Surprisingly, the two co-ligands Na^+ and aspartate induce opposite movements of HP2. We find that Na^+ binding to the apo state of the transporter opens the extracellular gate, while the subsequent binding of aspartate closes the gate. In addition, using voltage clamp fluorometry on the mammalian excitatory amino acid transporter EAAT3, we confirm that the opposite conformational changes of HP2 induced by Na^+ and amino acid substrates also occur in mammalian amino acid transporters. Our findings are consistent with HP2 comprising the extracellular gate of glutamate transporters, and that Na^+ binding opens and stabilizes the extracellular gate thereby allowing for amino acid substrate binding.