in larger amounts than does SDS across hairpin sequences. Pyrene excimer fluorescence data indicate that SPFO additionally retains more tertiary contacts vs. SDS for all hairpin sequences tested. Solubilization in SPFO therefore appears to favour protein-protein over lipid-protein interactions. Our overall results imply that the 'harshness' of a detergent is proportional to its protein sequence specificity upon binding, and consequent tendency not to disrupt intra-protein contacts during micelle formation.

Membrane Protein Function I

270-Pos

Anion Translocation in a Brush-Like Nanopore: Simulations of the Outer Membrnae Protein OprP

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The outer membrane protein OprP from *Pseudomonas aeruginosa* forms an anion-selective pore, especially selective for phosphate ions. The protein is homotrimeric, with each pore lined by three positively charged loops (L3, L5, and T7) folded into its lumen. OprP plays a key role in high-affinity phosphate uptake under the condition of phosphate starvation. To better understand the mechanism of phosphate-selective permeation, we employed three simulation techniques: (i) equilibrium molecular dynamics simulations (MD); (ii) steered MD (SMD); (iii) umbrella sampling to calculate a potential of mean force (PMF) for phosphate and chloride ions. The PMFs reveal a deep energy well midway along the OprP channel. Two adjacent phosphate-binding sites (W1 and W2), each with a well depth of ~8kT, are identified close to the L3 loop in the most constricted region of the pore. The transfer of phosphate between sites W1 and W2 is correlated with changes in conformation of the sidechain of K121, which serves as a 'charged brush' to facilitate phosphate passage between the two subsites. The PMF for chloride has also been computed and can be compared with that of phosphate. Our simulations suggest that OprP does not conform to the conventional picture of a channel with a relatively flat energy landscape for permeant ions, but rather resembles a membrane-inserted binding protein with a high specificity that allows access to a centrally located binding site from both the extracellular and the periplasmic spaces.

271-Pos

Regulation of Channel Function Due To Coupling With a Lipid Bilayer

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Regulation of membrane protein functions due to hydrophobic coupling with lipid bilayer is investigated. Binding energy between lipid bilayer and integral ion channel with different structures has been calculated considering 0th or 1st, 2nd, etc. order terms in the expansion of the screened Coulomb interaction V_{sc}(r)=integral of $d^3kExp\{i\mathbf{k}.\mathbf{r}\}V_{sc}(\mathbf{k})$ with V_{sc}(r) being the inverse Fourier transformation of the screened Coulomb interaction in Fourier space $V_{sc}(\mathbf{k})=V(\mathbf{k})(1+f(n,T)V(\mathbf{k}))^{-1}$ for bilayer thickness (d_0) channel length (l) mismatch (d_0-l) to be filled by none or single, double etc. lipids, respectively. $V(k)$ is the direct Coulomb interaction (in Fourier space) between channels and lipids on the bilayer, $f(n,T)=n/2k_BT$, n is the lipid density, T is absolute temperature and k_B is Boltzmann's constant. We find that the hydrophobic bilayer thickness channel length mismatch d_0-l induces channel destabilization exponentially while negative lipid curvature (c_0) linearly. Lipid charge appears with dominant effects in case of higher mismatch. Experimental parameters related to gramicidin A (gA) and alamethicin (Alm) channel dynamics in black lipid membranes inside NaCl aqueous phases are consistent with theoretical predictions. Our experimental results (with others) show that average gA channel lifetime decreases exponentially with increasing d_0-l but linearly with increasing negative c_0. The Alm channel formation rate and relative free energy profiles between its different conductance levels follow identical trends as predicted by our theoretical results. This study provides a general framework for understanding the underlying mechanisms of membrane protein functions in biological systems.

272-Pos

Properties of Liposomes With Complex Lipid Mixture

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Although the alpha-toxin from *S. aureus* was the first pore-forming toxin identified, its mode of interaction with membranes is still not fully understood. The toxin forms heptameric pores on cellular and artificial membranes. The observation that artificial membranes are permeabilized by this toxin indicates that no protein receptor is mandatory. Efficient permeabilisation is only possible in presence of cholesterol and sphingomyelin, which could be interpreted as a preference of the toxin for raft-like structures. However, the extent of oligomer formation as monitored by pyrene-fluorescence depends in a complex way on the lipid-composition of the liposomes which in our studies contain different amounts of eggPE, brainPS, eggSM and Cholesterol. Thus, we employed thin-layer chromatography in order to check whether the lipid composition as found finally in the liposomes correspond to the original mixture in chloroform. The results show that in case of extruded vesicles the deviation from the original mixture is not significant, but that in case of GUVs completely different relative amounts of the different lipid components can be obtained. Thus any comparison of liposome properties or toxin/liposome interactions between different liposome types has to be done very cautiously if these types of mixture are employed.
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273-Pos

Functional Reconstitution Into Liposomes of Purified Human RhCG Ammonia Channel

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Rh glycoproteins (RhAG, RhBG, RhCG) are members of the Amt/Mep/Rh family which facilitate movement of ammonium across plasma membranes. Changes in ammonium transport activity following expression of Rh glycoproteins have been described in different heterologous systems: yeasts, oocytes and eukaryotic cell lines. However, in these complex systems, a contribution of endogenous proteins to this function cannot be excluded. To demonstrate that Rh glycoproteins by themselves transport NH_3, human RhCG was purified to homogeneity and reconstituted into liposomes, giving new insights into its channel functional properties.

An HA-tag introduced in the second extracellular loop of RhCG was used to purify to homogeneity the HA-tagged RhCG glycoprotein from detergent-solubilized recombinant HEK293E cells. Electron microscopy analysis of negatively stained purified RhCG-HA revealed, after image processing, homogeneous particles of 10 nm diameter with a trimeric protein structure. Reconstitution was performed with sphingomyelin, phosphatidylcholine and phosphatidic acid lipids in the presence of the $C_{12}E_8$ detergent which was subsequently removed by Biobeads. Control of protein incorporation was carried out by freeze-fracture electron microscopy. Particle density was a function of the Lipid/Protein ratio. When compared to empty liposomes, ammonium permeability was increased two and three fold in RhCG-proteoliposomes, depending on the Lipid/Protein ratio (1/300 and 1/150, respectively). This strong NH_3 transport was reversibly inhibited by mercuric and copper salts and exhibited a low Arrhenius activation energy.

This study allowed the determination of ammonia permeability, showing that the apparent $Punit_{NH3}$ per RhCG monomer (around $1x10^{-3}$ $\mu m^3.s^{-1}$) is close to the permeability measured in HEK293E cells expressing a recombinant human RhCG ($1.60x10^{-3}$ $\mu m^3.s^{-1}$), and in red blood cells endogenously expressing RhAG ($2.18x10^{-3}$ $\mu m^3.s^{-1}$). The major finding of this study is that RhCG protein is active as an NH_3 channel and that this function does not require any protein partner.

274-Pos

Tracking Single Protein Translocation Complexes in the Membranes of Living Bacteria

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The Twin Arginine Translocation (Tat) system transports fully folded en sometimes even oligomeric proteins across the inner membrane of bacteria. Its mechanism is largely unknown. Remarkably, a stable translocation complex has not been observed. Instead, the three components of the system, i.e., TatA, TatB and TatC, are isolated from the membrane of Escherichia coli in various complexes of different sizes, which suggests that a complete and active Tat complex is formed only transiently. We have used single particle tracking in living bacteria to gain more insight into the dynamics of the Tat proteins. TatA has been genetically fused tot enhanced Green Fluorescent Protein (eGFP). Living bacteria expressing low levels of TatA-eGFP have been immobilized on glass slides and imaged with a sensitive wide-field fluorescence microscope. Mobile fluorescent spots are observed, and their intensity and location have been tracked by fitting a 2D Gaussian function to successive frames. Analysis of the data shows that diffusion of TatA-eGFP is heterogeneous, and that the average diffusion coefficient of fluorescent TatA particles decreases when excess substrate is expressed. When the electrochemical potential, which is known to drive protein transport via the Tat system, is removed the diffusion coefficient of TatA-eGFP increases again. The latter suggests