Their structural characterization by circular dichroism and fluorescence emission revealed a structure entirely analogous to that of SP-B purified in organic solvents. However, detergent-purified SP-B showed a much more oligomeric structure, as analyzed by blue-native electrophoresis, analytical centrifugation or electron microscopy. This SP-B complex has been reconstituted into surfactant phospholipids and the resulting lipid/protein complexes showed in the captive bubble surfactometer (CBS) a similar surface active behaviour than provided by SP-B purified using the classic chloroform/methanol-based method.

3176-Pos Board B37

Structural Flexibility of the Cytoplasmic Domain of Flagellar Type III Secretion Protein FlhB is Important for the Function of the Protein

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Bacterial flagellum is a large complex organelle made up of more than 30 different proteins. Most of the flagellar proteins are localized outside of the cell and are exported across the cytoplasmic membrane by the flagellar type III secretion system. The protein export is highly regulated. Membrane protein FlhB plays a key role in this regulation. The protein consists of two domains: a hydrophobic N-terminal part ($FlhB_{TM}$), which is predicted to have four transmembrane helices, and a C-terminal cytoplasmic domain ($FlhB_C$). Homologues of FlhB were found in all bacterial type III secretion systems. Sequences of these proteins are highly conserved suggesting that their function is also similar.

In this study we have compared properties of FlhB from two organisms: *Salmonella typhimurium* and *Aquifex aeolicus*. *Salmonella* and *Aquifex* FlhB share 32% sequence identity. However, these proteins are evolutionarily very distant. Comparison of the two proteins may provide us with additional information about functionally important regions of FlhB.

We have substituted *flhB* gene in *Salmonella* by *flhB* of *A. aeolicus* or by chimera gene encoding hybrid FlhB composed of the $FlhB_{TM}$ of *S. typhimurium* and $FlhB_C$ of *A. aeolicus*. Then we analyzed motility of the mutants on soft agar plates. Although all mutants showed some motility, they were substantially less motile than wild-type cells. We have found several spontaneous mutations in C-terminal part of FlhB that resulted in enhanced motility. To understand the effect of the mutations we have solved $FlhB_C$ structures from both organisms: *Salmonella* and *Aquifex*. We have also determined secondary structure and stability of the mutated $FlhB_C$. Based on our findings we suggest that conformational flexibility is important for FlhB function.

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Exploring the Biophysical Properties of Human Uncoupling Proteins: A Search for their Physiological Roles in the Central Nervous System

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Expressed in brown adipose tissues (BAT), uncoupling protein-1 (UCP1) facilitates proton transport across the inner mitochondrial membrane, reducing the membrane potential and rate of ATP synthesis. The excessive proton flux carried by UCP1 produces heat in BAT. Three other UCP homologues (UCP2, UCP4 and UCP5) are expressed in the central nervous system (CNS), but their physiological functions are not well understood. The goal of this study is to explore the biophysical properties of neuronal UCPs reconstituted in liposomes to gain an insight into the specific roles of these proteins in the CNS. The three neuronal UCPs were recombinantly expressed, purified and reconstituted in lecithin liposomes (with and without the supplement of 2.5 mol% cardiolipin (CL)). Ion transport assays (proton and chloride) for reconstituted UCPs were developed using anion-sensitive fluorescent probes. All neuronal UCPs displayed proton transport across the membrane with characteristics similar to the archetypical protein UCP1, which is activated by fatty acids and inhibited by purine nucleotides. UCP2 and UCP4 showed high alpha-helical contents in liposomes and conducted chloride. Ion transport of UCPs 4 and 5 was reported for the first time in this study. In addition, it was observed that the mitochondrial lipid CL induced changes in conformation and ion transport properties of reconstituted UCPs. A hypothetical interaction mechanism of UCPs and CL was drawn from the experimental results and molecular modelling. Overall, this study provides the groundwork on the conformation and ion transport properties of neuronal UCPs in liposomes, and emphasizes the crucial role of cardiolipin in UCPs' structure and function. Understanding the structure-function relationships of neuronal UCPs will be essential in shedding light on their potential roles in protection against neurodegenerative diseases in the CNS.

3178-Pos Board B39

On the Role of CBS Domains in Osmoregulatory ABC Transporters

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Cell volume regulation is an essential function of any cell to overcome the consequences of osmotic stress. Under hyper-osmotic conditions, a cell accumulates or synthesizes compatible solutes to prevent shrinkage and ultimately plasmolysis. The osmoregulatory ATP-binding cassette (ABC) transporter OpuA responds to hyper-osmotic stress by taking up the compatible solute glycine betaine. The CBS module of OpuA in conjunction with an anionic membrane surface acts as sensor of internal ionic strength, which allows the protein to respond to osmotic stress. We now show by chemical modification and cross-linking studies that CBS2-CBS2 interface residues are critical for transport activity and/or ionic regulation of transport, whereas CBS1 serves no functional role. We establish that Cys residues in CBS1, CBS2, and the nucleotide-binding domain (NBD) are more accessible for cross-linking at high than low ionic strength, indicating that these domains undergo conformational changes when transiting between the active and inactive state. Structural analyses (light-scattering, circular dichroism and NMR) suggest that the CBS module is largely unstructured. Moreover, we could substitute CBS1 by a linker and preserve ionic regulation of transport. These data suggest that CBS1 serves as linker and the structured CBS2-CBS2 interface forms a hinge point for ionic strength-dependent rearrangements that are transmitted to the NBD and thereby affect translocation activity.

3179-Pos Board B40

Energy Transduction Between Membranes: Characterization of the Interaction Between TonB and its Cognate Transporters

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In gram-negative bacteria, essential nutrients such as iron and cobalamins are too large to diffuse through porins in the outer membrane and therefore require an active transport mechanism for their uptake. Since there is no source of energy at the outer membrane, this is a complicated task that involves coupling of the outer membrane transporter to the protonmotive force across the cytoplasmic membrane via the proteins ExbB, ExbD and TonB. Presumably, ExbB and ExbD utilize this gradient to conformationally energize TonB, which spans the periplasm and associates with the transporters in the outer membrane to deliver the energy that is required for transport. In this study, we use fluorescence anisotropy to measure the affinity of a TonB fragment (lacking the transmembrane domain) for three of its cognate transporters–BtuB, FhuA, and FecA. Additionally, we determine the effect of substrates, Colicin E3, and transport-defective mutations to the coupling motif (Ton box) on the affinity of this interaction. The oligomeric state and structure of TonB in solution and bound to the transporters will also be discussed. These results have implications for the development of new antibiotics that interfere with the TonB-transporter interaction.

3180-Pos Board B41

Creating a Proteoliposome Assay for Single Photosystem I Activity Assessment

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Photosystem I (PSI) is a unique photoelectronic nanomachine that produces the largest negative potential in nature, and principally sets the global enthalpy amount in all lifeforms [1].

Single molecule measurements are ideally suited to elucidate molecular level details underlying PSI activity, that remain masked in conventional ensemble PSI activity assays. These assays are usually carried out on native thylakoid membranes, containing different sizes and compositions of proteins and lipids, or on PSI solubilised in detergent, thus yielding an uninformative average activity.

Here, we have employed our recently developed arrays of surface tethered single liposomes [2] on reconstituted single PSI, allowing us to monitor its activity at the single molecule level and in a massive parallel manner [3]. Liposomes constitute an ideal 3D scaffold to spatially confine single PSI in a native like environment, and can efficiently encapsulate the prefluorescent electron

acceptor, resazurin [4], that upon reduction by PSI becomes highly fluorescent, thus directly yielding single PSI activity. Our studies allow us for the first time to correlate membrane characteristics (lipid composition, curvature, phase state, etc.), to regulation of PSI activity studied on the single molecule level.

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3181-Pos Board B42

A Revised View on Cellular CO2 Transport Mechanisms: Biophysics, Physiology and Genomics of Aquaporin-Facilitated CO2 Transport in Plants

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It is a general opinion that the Meyer-Overton correlation determines biomembrane transport of hydrophobic molecules. This so called ''Overton rule'' says that the easier it is for a chemical to dissolve in a lipid the easier and faster it will be transported into a cell. In medical science for example, this passive transport is crucial for the effective delivery of many pharmaceutical agents to intracellular targets. The prediction also concerns CO2 as a hydrophobic molecule. Certainly, the membrane diffusion of CO2 is of critical importance for bacteria, animal- and plant cells. In contrast to many other organisms, plants require CO2 and its availability at the site of CO2 fixation limits the rate of net photosynthesis. In this regard, plants provide an excellent system to study CO2 transport mechanisms. Findings will be presented, which question the applicability of Overton's rule to specific plant CO2 transport processes. It could be demonstrated that the function of specific membrane proteins, i.e. distinct aquaporins, increased CO2 transport rates. The experiments were performed on synthetic membranes and cell based systems as well as plant tissues and complete plants. Techniques from biophysics, cell biology, molecular biology and plant physiology were employed and it was found that in the analyzed systems CO2 transport rates were limited by the function of these aquaporins. The results could be interpreted in a way that supports alternative cellular CO2 transport mechanisms and a modified model of cellular surfaces. If the findings were of general validity and not specific for plants, our view on diverse transport processes in many living organisms from all kingdoms could be modified.

3182-Pos Board B43

Determinants of Lipid Mixing Membrane Fusion by HIV gp41

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A critical step in HIV infection involves membrane fusion between the enveloped virion and the target cell plasma membrane, catalyzed by the viral membrane protein gp41 at or near physiologic pH. Specific global gp41 conformations (i.e. six-helix-bundle and coiled-coil) and specific regions (i.e. fusion peptide) are implicated in steps of fusion catalysis. Our goal is to elucidate the molecular mechanism of gp41 catalyzed membrane fusion. One challenge to biophysical analysis of structure and function in hydrophobic gp41 is solubility. Low pH (~3.0) is utilized to enhance protein solubility by significantly increasing overall positive charge. Interestingly, the cationic six-helix-bundle region enhances/inhibits lipid mixing of anionic vesicles at low/neutral pH, which correlates with close association (~5Å) between membrane headgroup phosphates and a large/small proportion or population of helical bundles based on solid-state NMR analysis. The apolar trimeric fusion-peptide region contributes to lipid mixing and is predominantly β-sheet (~75%) with a large fraction of β-strands located close to (~5-7Å)/far from (>~10Å) membrane headgroup phosphates at residues Ala-1 and Ala-14/Leu-7 and Met-19 at low pH. Swapping to neutral pH does not affect fusion-peptide conformation, but does lower the fraction of β-strands close to the membrane surface. Partial shedding of fusion-peptides from membranes, poor protein solubility, and protein lipid mixing inactivity at neutral pH are reversed upon pH swap back to low, indicating irreversible aggregation is not occurring at neutral pH. Our solid-state NMR findings indicate that abrogation of lipid-mixing fusion function by gp41 in six-helix-bundle conformation at the physiologic pH of viral fusion is determined primarily by stearic and electrostatic barriers to close membrane apposition imposed by the six-helix-bundle region, and minimally by membrane location or conformation of the fusion-peptide region.

3183-Pos Board B44

Time-Resolved UV-Visible Studies of Rhodopsin Provide Experimental Test of Flexible Surface Model for Lipid-Protein Interactions

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Time-resolved UV/Visible absorbance of the retinal chromophore of rhodopsin [1] delivers vital information about key GPCR activation steps in a membrane lipid environment [2]. Following rhodopsin photoexcitation, a minimum of four intermediates equilibrate on the millisecond-time scale [2,3]. The first equilibrium is between the protonated Schiff base (PSB) species Meta I_{480} and the deprotonated SB species Meta II_a and is pH independent. A second equilibrium entails spectrally silent conversion of Meta II_a to the Meta II_b substate. The final step is protonation of Glu134 of the ERY sequence in Meta II_b to yield Meta II_bH^+ whose pK_a characterizes the acid-base equilibrium [3]. Absorbance measurements on the microsecond-to-hundred millisecond-time scale allowed us to study effects of POPC, DOPC, or DOPC/DOPE mixtures on the first equilibrium constant K_1 and on the pK_a of the final equilibrium. Results were analyzed by singular-value decomposition and were fit by a linear combination of temperature-dependent basis spectra. Notably an increase in K_1 was discovered due to either PE head groups or increased acyl chain unsaturation, whereas little change in p$K_a$was evident. According to the flexible-surface model (FSM) rhodopsin becomes a sensor of negative spontaneous (intrinsic) curvature upon light activation [4]. Competition between the curvature elastic energy and the hydrophobic mismatch at the proteolipid boundary explains the influences of lipid-protein interactions [4]. Both the lipid acyl chains and polar head groups affect the Meta I-Meta II transition, revealing how protein energetics are affected by material properties of the lipid bilayer.

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3184-Pos Board B45

Dynamics and Oligomerization Tune Seven-Transmembrane Protein Function: Studies Based on Proteorhodopsin

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Seven-transmembrane (7TM) proteins have diverse and important functions, ranging from signaling receptors to ion pumps. They share a reversible switching property, epitomized by the solar-powered microbial proton pump Proteorhodopsin (PR), which uses light energy to facilitate transport by a conformational ''switch''. Here, we use PR as a model to capture the elusive details of activation and oligomerization necessary for the function of physiologically important membrane proteins.

We have preliminary spectroscopic evidence that suggests altered photocycle kinetics and shifted pKa values for hexameric and monomeric forms of detergent-solubilized PR. These findings prove that PR function is tuned by self-association, and we apply magnetic resonance together with functional assays of proton transport to further understand dynamics changes that occur upon oligomerization. Our unique magnetic resonance techniques of electron paramagnetic resonance (EPR) and dynamic nuclear polarization (DNP) provide insight into the protein segment mobility and local hydration water dynamics of an amino acid residue spin-labeled with nitroxide-based radicals.

Using these methods, we have found that PR's third cytoplasmic (E-F) loop is a short α-helical segment that experiences conformational change upon photoactivation. This structure is a common motif to the non-homologous G-protein coupled receptor bovine rhodopsin (Rh), where it is a docking point for a signal G-protein. Towards understanding how function hinges on dynamics, we developed a PR-Rh chimera by replacing the E-F loop of PR with the corresponding loop of Rh. The chimera successfully expresses and maintains optical properties. We evaluate its capability to activate the G-protein transducin, and apply EPR and DNP to obtain unique information about the biophysics of receptor/G-protein interactions. By controlling the oligomeric form of the PR-Rh chimera, we measure any changes in G-protein activation caused by varying the amount of receptor-receptor interactions.

3185-Pos Board B46

Molecular Mechanism of Activation of IRE1α Cytosolic Domain by Palmitate

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Inositol-requiring enzyme 1α (IRE1α) is an ER (endoplasmic reticulum) transmembrane containing two enzymatic activities, a Ser/Thr protein kinase and an