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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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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 car-

diolipin 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 Bert Poolman.

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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