faith of a cell are members of the Bcl-2 protein family interacting with the mitochondrial outer membrane (MOM) and modulating its permeability. For the longest time MOM forming lipids have been seen as structural building blocks without any mechanistically importance in apoptosis. This view changed in recent years after evidence for the direct involvement in apoptotic events of oxidized phospholipids (OxPls) formed under intracellular stress has been provided. Since the presence of OxPls strongly influences the equilibrium of pro-apoptotic Bax protein towards its membrane bound state, we investigate their role in the Bax induced step of membrane pore formation and the undergoing structural changes of Bax protein during this event.

We therefore created cell-free MOM mimicking liposome systems who resemble the cellular situation prior and upon oxidative stress. By using differential scanning calorimetry accompanied by 1H, 13C and 31P solid state MAS NMR spectroscopy we could gain insights into the hydrophilic interface region of the membranes as well as their hydrophobic fatty acid regions. Upon incorporation of OxPls both methods revealed drastic changes in the dynamics of the membranes. Additionally we could relate these perturbations caused by lipid oxidation to an increased membrane affinity of pro-apoptotic Bax protein, presumably aiding Bax membrane penetration necessary for successful pore formation. Currently we are investigating the structural changes of Bax protein upon membrane insertion by solid state MAS NMR spectroscopy to gain mechanistically insights in the process of MOM pore formation. First 15N NMR suggest the observation of a Bax-lipid assembly. Structural studies with fully labeled Bax protein are in progress.

314-Pos Board B94

Identification of Conformation Specific Binder for the NA^+/Galactose Transporter

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Na^+/galactose transporters (SGLTs) are integral membrane proteins, which co-transport Na^+ with sugars from the periplasmic space into the cytoplasm. According to the alternating access model for secondary active transporters, these proteins alternate between outward and inward-facing conformations during the transport cycle. The currently available structures from a bacterial homolog of SGLT from *Vibrio parahaemolyticus*, (vSGLT) are in the substrate-bound inward-occluded and the substrate-free inward-open conformations. Despite much effort, structures of the outward conformations remain elusive.

Isolation of distinct conformations of transporter is a major obstacle for X-ray crystallography due to their conformational heterogeneity. Crystallization chaperones based on various protein scaffolds have emerged as a promising tool to increase the crystallization probability of a selected target protein. Sso7d is a highly stable binding protein derived from the hyperthermophilic archaeon *Sulfolobus solfataricus*. It has a versatile scaffold for generating binding protein for a wide spectrum of targets. Sso7d-derived proteins are far easier to produce in bacteria and due to their small size, they are capable of targeting areas that are not accessible to standards antibodies.

To find binders for the SGLT transporter, we screened an Sso7d-based yeast display library using flow cytometry. After several screening steps, we isolated and purified clones that bind the SGLT protein. We are currently attempting to crystallize a complex of the binder with SGLT. Here we present the characterization of Sso7d-binder from the yeast display library using a two-step procedure involving magnetic and FACS-based screening. The micro-molar interaction between Sso7d-binder and vSGLT was determined by analytical gel filtration, SDS-PAGE, microscale thermophoresis and isothermal titration calorimetry. We anticipate this binder will assist in crystallization of the vSGLT protein. Future screening will utilize transport-impaired mutants to select for an outward-facing conformation, which will provide mechanistic insights of the transport mechanism.

315-Pos Board B95

NMR Solution Structure and Extracellular Loop Dynamics of the Outer Membrane Protein OprG of Pseudomonas Aeruginosa Explain Transport of Small Amino Acids

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Pseudomonas aeruginosa is an opportunistic human pathogen that is responsible for a growing number of nosocomial infections and the main cause of death in patients with cystic fibrosis. The structure of one of its outer membrane proteins (OprG) has been solved by x-ray crystallography and revealed a tall 8-stranded β-barrel that extends far into the extracellular space with a lumen lined with hydrophobic residues (Touw et al, PLoS ONE 5:15016[2011]). An interesting feature of that structure is a lateral proline-rich opening in the barrel wall near the membrane interface that has been suggested to function as a gate for small hydrophobic molecules that might subsequently diffuse across the bilayer of the outer membrane.

In order to test this hypothesis we re-determined the structure of OprG in DHPC micelles by NMR. We show that the β-barrel has substantially shorter strands than in the crystal structure and longer extracellular loops without a defined lateral gate. To further investigate the role of the prolines in the proposed gate, we mutated them to alanines and determined the structure of the P92A mutant. Introduction of the P92A mutation resulted in an asymmetric elongation of the beta-barrel and changed the loop dynamics and the hydrogen-bonding pattern in the barrel-to-loop transition regions. As shown on a separate poster by PS, IK, and LKT, the physiological substrates of OprG are small amino acids and the P92A mutation aborts small amino acid transport.

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Molecular Basis for the Interaction of Lipopolysaccharide with Outer Membrane Protein OprH from Pseudomonas Aeruginosa

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Pseudomonas aeruginosa is an opportunistic pathogen that infects cystic fibrosis and immunocompromised patients. The impermeability of the P. aeruginosa outer membrane contributes substantially to the notorious antibiotic resistance of this human pathogen. This impermeability is partially imparted by the outer membrane protein OprH. The function of OprH is to provide increased stability to the outer membranes of P. aeruginosa by directly interacting with LPS molecules. The NMR solution structure of OprH reveals an eight-stranded β-barrel with four extracellular loops of unequal size (Edrington et al, J. Biol. Chem. 286:39211[2011]). Based on NMR chemical shift perturbations observed upon the addition of LPS to OprH in lipid micelles, we concluded that the interaction is predominantly electrostatic and localized to charged regions near upper rims of the barrel and the two shortest loops. Using an enzyme-linked immunosorbent assay (ELISA) we discovered that the intact secondary structure is necessary for OprH to interact with LPS, and that the interaction occurs mainly through the charged side chains of K70, R72, K103 and R113. Despite detailed analysis of NMR spectra upon the addition of LPS to OprH, we found no evidence for an interaction of the hydrophobic side-chains of I, L and V with lipid A of LPS. The results of this study provide a more definitive molecular model for interactions between OprH and LPS and offer new insight into protein-lipid interactions that likely contribute to the antibiotic resistance during P. aeruginosa infections.

317-Pos Board B97

Symmetry and Size of Membrane Protein Polyhedral Nanoparticles

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The biologically relevant structures, and resulting functions, of many membrane proteins depend critically on their lipid bilayer environment. Yet, determination of membrane protein structure in native lipid bilayer environments and in the presence of functionally relevant transmembrane gradients has largely remained elusive. Recent experiments on membrane protein polyhedral nanoparticles (MPPNs) [T. Basta, et al. PNAS 111, 670 (2014)] present an exciting step towards overcoming this challenge. In these experiments, lipids and membrane proteins were observed to self-assemble into polyhedral bilayer vesicles with a well-defined 3D polyhedral arrangement of membrane proteins, which permits structural analysis using electron cryo-tomography, with the membrane proteins embedded in native lipid bilayer environments and the closed surfaces of MPPNs allowing establishment of transmembrane gradients. However, to allow high-resolution studies, MPPNs must exhibit controlled symmetry and size. To aid the development of MPPNs as a novel method for structural analysis of membrane proteins, we develop minimal molecular and thermodynamic-mechanical models of MPPNs which capture the physical mechanisms underlying the key experimental features of MPPNs. Our thermodynamic-mechanical model of MPPNs builds on previous work on membrane budding and viral capsid formation to describe the internal energy of MPPNs in terms of protein-induced lipid bilayer bending deformations and spherical packing defects. We confirm the assumptions underpinning our thermodynamic-mechanical model through Monte Carlo simulations of our minimal molecular model. We analytically solve our thermodynamic-mechanical model to predict the phase diagram of MPPNs, which we show to be in excellent quantitative agreement with experiments of MPPNs. Our theoretical approach thus establishes a

quantitative link between the observed symmetry and size of MPPNs and their molecular composition and external environment. We suggest how these insights may be utilized to optimize MPPN stability, symmetry, and size, and to produce MPPNs for general membrane proteins.

318-Pos Board B98

Structural Basis for Phosphatidylinositol-Phosphate Biosynthesis

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Phosphatidylinositol is critical for intracellular signaling and anchoring of carbohydrates and proteins to outer cellular membranes. In eukaryotes, phosphatidylinositol-based lipids play important roles in numerous aspects of signal transduction and in the anchoring of glycosylphosphatidylinositol (GPI) linked proteins to the membrane. In prokaryotes, phosphatidylinositol (PI) is produced by mycobacteria, as well as some other bacterial genera, where it is required for the biosynthesis of key components of the cell wall, such as the glycolipids lipomannan and lipoarabinomannan, which are tethered to the membrane via a common PI anchor. In *Mycobacterium tuberculosis,* these glycolipids function as important virulence factors and modulators of the host immune response.

The defining step in phosphatidylinositol biosynthesis is catalyzed by CDP-alcohol phosphotransferases (CD-APs), transmembrane enzymes that use CDP-diacylglycerol as donor substrate for this reaction, and either inositol in eukaryotes or inositol phosphate in prokaryotes as the acceptor alcohol. In prokaryotes, this reaction is catalyzed by the CDP-AP phosphatidylinositol-phosphate synthase (PIPS) to yield phosphatidylinositol-phosphate, which is in turn dephosphorylated by an as yet uncharacterized enzyme to PI. Given its essentiality in mycobacterial viability—conditional knockouts prove fatal—and its divergence from the eukaryotic counterpart, PIPS can be considered a promising target for anti-tuberculosis drugs.

We have determined structures of PIPS from *Renibacterium salmoninarum,* with and without bound CDP-diacylglycerol to 3.6 and 2.5Å resolution, respectively. These structures reveal the location of the acceptor site, and the molecular determinants of substrate specificity and catalysis. Functional characterization and mutagenesis of the 40%-identical PIPS orthologfrom *Mycobacterium tuberculosis* supports the proposed mechanism of substrate binding and catalysis. This work therefore provides a structural and functional framework to understand the mechanism of phosphatidylinositol-phosphate biosynthesis.

319-Pos Board B99

Sans Studies of Bacteriorhodopsin Incorporation and Crystallization in Cubic Phase

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The direct crystallization of membrane proteins from lipidic mesophases has become an increasingly important, and even routinely used, tool in the field of membrane protein structural biology. Despite the method's practical successes, however, the exact molecular mechanisms for crystallization remain poorly understood. It has been proposed that protein molecules first align in transient, locally lamellar regions of lipid, forming small 2D crystals that initiate growth. These crystals could then stack to form the 3D crystal, which is fed laterally by lamellar-phase membrane extensions from the bulk cubic phase. Nevertheless, the initial formation of 2D crystal ''patches'' and initiation of lamellar transition has not been experimentally observed. Other mechanisms of crystal formation can also be envisioned, and the possibility of multiple crystallization mechanisms exists. A clear understanding of these mechanisms would allow for the rational optimization of crystallization conditions for different proteins, including the use of different lipids and additives.

Using Small-Angle Neutron Scattering (SANS), we have begun a systematic study of cubic phase crystallization using the membrane protein Bacteriorhodopsin (bR) in monoolein cubic phase as a model system, alone and in the presence of precipitants. SANS with contrast variation is uniquely suited for the study of proteins in cubic phase, since the scattering contribution of the large excess of lipid can be eliminated, allowing the scattering from embedded protein to be directly measured without interference. This allows us to study bR embedded in the monoolein cubic phase.

320-Pos Board B100

Statistical Learning and Docking Recover the Reaction Coordinates of a GPCR

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GPCRs comprise one-third of targets of all FDA-approved drugs. Molecular dynamics (MD) simulations of GPCRs can contain over 60,000 atoms, counting for over 180,000 degrees of freedom. The technique described here reduces the dimensionality of GPCR MD simulations through a combination of unsupervised and supervised learning. In particular, time-structure Independent Component Analysis (tICA) [2,3] and molecular docking are used complementarily to determine reaction coordinates relevant to agonist binding and receptor activation.

321-Pos Board B101

Structural Characterization of the Bacterial Succinate/Acetate Proton Symporter SatP in Lipid Bilayer Membranes

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Bacterial succinate-acetate/proton symporter SatP is an integral membrane protein, responsible for transport of the negatively charged carboxylic acids across the lipid bilayer. The 20 kDa protein consist of six glycine-rich transmembrane helices, connected by loops, and forms hexameric aggregates. We sought to elucidate the structure and mechanism of this bacterial transporter, as this class is underrepresented in the protein databank, with the goal to learn the mechanism of ligand transport that can be used as a basis for homologous eukaryotic transporters.

The protein has been expressed in *E. coli* using minimal media and labeled with NMR-active $^{13}C/^{15}N$ nuclei either uniformly, or on a amino acid specific basis. Reconstitution in lipid bilayers yielded the active form of the transporter, which was subjected to magic angle spinning multidimensional NMR experiments, yielding well resolved spectra of the backbone and side chain resonances. Using through bond correlation experiments, we have assigned the majority of the backbone and performed initial structural characterization using the dihedral angles derived from TALOS-N with N, CA and C' chemical shifts. Our current focus is on completing the spectral assignments, which would allow generating the first structural models of this transporter.

322-Pos Board B102

Conformation of the Membrane-Integrated Functional State of Anti-Apoptotic BCL-XL

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BCL-XL is a major suppressor of apoptosis and a prominent member of the BCL-2 family. It is expressed in a variety of tissues and cell types and overexpressed in many tumors where it acts to promote tumor cell survival, tumor formation and tumor resistance to chemotherapy. Structure and activity studies have focused on truncated forms of the protein without the hydrophobic C-terminus that is essential for mediating the protein's association with intracellular membranes and cytoprotective activity. Hence, while the cytosolic state of the protein is relatively well understood, molecular information about the membrane-bound state is limited. We have used solution and solid-state NMR, together with isothermal titration calorimetry, to examine the structure and ligand binding activity of membrane-integrated BCL-XL, including its complete hydrophobic C-terminus. The fraction of BCL-XL expressed in *E. coli* as insoluble inclusion bodies can be isolated, purified and refolded into phospholipid bilayers. Here we describe the conformation of this membrane-bound state as a soluble globular domain anchored by a transmembrane C-terminal [[Unsupported Character - Symbol Font α]] helix. Membrane-bound BCL-XL binds a BID BH3 peptide with affinity similar to or slightly greater than the C-terminal truncated form. Implications for apoptosis from the conformation the membrane-integrated BCL-XL are discussed.

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