Rhomboid intramembrane proteases catalyze the transmembrane domain cleavage of single-pass membrane proteins. This activity is crucial for the activation of epidermic growth factor receptor ligands Gurken, Keren and Spitz in D. melanogaster. How rhomboids recognize their substrates and select which peptide bond to cleave is not understood. We have studied the substrate specificity and peptide bond selectivity of purified rhomboids from E. coli, P. aeruginosa, D melanogaster and H. sapiens using chimeric substrates containing the transmembrane domains of Gurken, Keren and Spitz. Analysis of the proteolytic products by mass spectrometry reveals that cleavage occurs in the membrane water interface at sites that are shared by both eukaryotic and prokaryotic rhomboids. Mutagenesis of the substrates reveals a helical amino acid motif that is crucial for substrate recognition and peptide bond selection. With insight from computational data a model for the substrate-enzyme complex will be presented.

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Biophysical Properties of Transmembrane Segment 6 of E.coli MntH Transporter

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The Natural Resistance-Associated Macrophage Protein (Nramp) family of secondary active divalent metal ion transporters plays an important role in a variety of biological processes, such as metal ion homeostasis, in virtually all living organisms. Due to its structural and functional homology with eukaryotic Nramps, the E. coli transporter MntH (Proton-dependent Manganese Transporter) represents a prototypic model to advance understanding of structure-function relationship in Nramp family. Synthetic peptides corresponding to the transmembrane (TM) segments of membrane proteins could serve as a suitable alternative model for studying the structure and interaction of the membrane protein TM domains with biological membranes. In this study the synthetic peptide corresponding to the sixth transmembrane segment (TMS6) of E. coli MntH and its two mutants, in which the His211 residue was substituted by arginine or alanine, were used. TMS6 was previously shown to contain two functionally important histidine residues. The H211A mutation preserves bacterial sensitivity to metal ions and facilitate H+ uptake in the presence of metal ions. In contrast, H211R does not induce metal sensitivity (1,2). The secondary structures of TMS6 and its mutants were determined in model membranes and membrane-mimicking organic environments, using CD spectroscopy. The conformation of the peptides exhibited ordered α and β conformations in these milieus. Furthermore, patch clamp measurements demonstrated that TMS6 was able to form multi-state ion channels in the presence of manganese as a physiological substrate of MntH. The mutant H211R does not show any channel-like activity and with the mutant H211A the ion channel activity was rarely observed.

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Solid State NMR of Membrane Proteins: Towards Complex Structural and Functional Information for Bacterial ABC Class Importers

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Despite the wealth of protein structural data available today, membrane protein structural characterization continues to pose a significant challenge in structural biology. Choosing the membrane mimetic is a challenge and often detergent micelles are employed. However, detergents are prone to induce distortions in the protein structure. An emerging technique, solid state nuclear magnetic resonance (NMR), provides a path to structural characterization using an environment similar to the native one, liquid crystalline lipid bilayers.

Static solid state NMR experiments on proteins determine the orientation of the peptide planes with respect to the magnetic field. All ^{15}N-^{1}H dipolar couplings and anisotropic ^{15}N chemical shifts observed in two dimensional separated local field experiments (PISEMA) lie within a butterfly shape in the spectrum. Special patterns called PISA wheels arise for uniformly aligned protein samples which directly reflect the orientation of protein secondary structure. Using these wheels the tilt angle of each helical axis from the magnetic field and membrane normal can be determined without complete structure determination. Consequently, a single data set allows for characterization of secondary structure in the membrane mimetic as well as providing a set of high resolution peptide plane orientations that can be used directly in structural refinement.

Uniform alignment has been achieved for multiple proteins in our laboratory. Still, studies on larger membrane proteins are required to make solid state NMR a generally applicable technique for membrane protein structure characterization. One excellent example is SugAB, the transmembrane domain of an ABC importer from *Mycobacterium tuberculosis.* These transport proteins contain at least 10 transmembrane helices. This makes SugAB an excellent target for determining the utility of solid state NMR to structurally characterize large membrane proteins.

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Re-examination of the Role of the Amino-Terminus of SecA in Promoting Its Dimerization and Functional State

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The SecA nanomotor promotes protein translocation in Eubacteria by binding both protein cargo and the protein-conducting channel and undergoing ATP-driven conformation cycles that drive this process. Conflicting reports exist as to whether SecA functions as a monomer or dimer during this dynamic process.Here we re-examine the role of amino and carboxyl termini of SecA in promoting its dimerization and functional state by examining three secA mutants and their respective proteins: SecAD8 lacking residues 2-8, SecAD11 lacking residues 2-11, and SecAD11/N95 lacking both residues 2-11 and its carboxyl-terminal 70 residues. We demonstrate that whether or not SecAD11 or SecAD11/N95 was functional for promoting cell growth depends solely on their vivo levels that appear to govern residual dimerization. Cell fractionation revealed that SecAD11 and SecAD11/N95 were still proficient in membrane association, although they were reduced in the formation of integral membrane SecA. The presence of a modestly higher level of SecAD11/N95 in the membrane and its ability to form dimers as detected by chemical crosslinking were consistent with the higher secA expression level and better growth property of this mutant compared to secAD11. Biochemical studies showed that SecAD11 and SecAD11/N95 displayed identical dimerization defects, while SecAD8 was intermediate between these proteins and wildtype SecA. Furthermore, both SecAD11 and SecAD11/N95 were equally defective in their translocation ATPase specific activity. Our studies show that the non-essential carboxyl-terminal 70 residues of SecA play no role in its dimerization, while increasing truncation of the amino-terminal region of SecA from 8 to 11 residues results in an increasing defect in SecA dimerization and poor in vivo function unless highly overexpressed and also clarify a number of conflicting reports in the literature and support the essential nature of the SecA dimer.

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Substrate Selectivity in Adic, an E. Coli Inner Membrane Arginine-agmatine Antiporter

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AdiC is a membrane antiporter that transports arginine and its decarboxylation product agmatine across E. coli inner membrane. It plays a key role in the arginine-dependent extreme acid resistance. We overexpressed AdiC in E. coli and reconstituted the purified protein into liposomes. A series of arginine analogs were tested on the transporter. The permeability sequence is as follows: arg / agm > 1,5-diaminopentane, 1,6-diamino-hexane, 1,4-diaminobutane >> argininamide, lysine, ornithine, canavinine. Kinetic analysis results are K_m~ 1mM for 1,5-diaminopentane, ~3 mM for argininamide and lysine, ~15 mM for canavanine.

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Identification of Functionally Important Sites within the Cysteine-Free Inner Membrane Transferase Protein ArnT

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The bacterial inner membrane protein ArnT confers resistance to the antibiotic polymyxin in *Salmonella typhimurium* and *Escherichia coli* through the modification of lipid A, the major component of the outer surface of Gram-negative bacteria. ArnT transfers a neutral aminoarabinose moiety (L-Ara4N) onto the negative phosphate group(s) of lipid A, which significantly reduces the surface charge of these bacteria and thus prevents cationic peptides such as polymyxin from electrostatically recognizing and killing the bacteria. We have previously reported the first expression, purification and functional analysis of ArnT from *S. typhimurium*, and our studies showed that ArnT is highly α-helical and described a new *in vivo* functional assay. In this continuation of the characterization of the ArnT protein, we used the cysteine-specific maleimide-PEG_{5000} to demonstrate that all eight of the native cysteines in *S. typhimurium* ArnT are in the reduced form and therefore not involved in disulfide bonds. In addition, we created a cysteine-free protein that is structurally and functionally intact as determined by circular dichroism and the results of the new *in vivo* growth