brane voltage. Several crystal structures of the C-terminal truncated transmembrane domain of KcsA have provided extensive information on ion selectivity and permeation, and have contributed to our understanding of the gating mechanism of the channel. Although spectroscopic evidence clearly shows that the 40-residue C-terminus forms four-helix bundle that projects to the cytoplasm, full-length (FL) KcsA has so far remained refractory to high-resolution crystallographic approaches. Here, we have generated novel nM affinity Fabs against FL-KcsA from a phage display library. These Fabs have been used as crystallographic chaperones to generate high quality crystals of FL-KcsA, diffracting to a resolution of 3.7 Å. The structure of the FL-KcsA-Fab complex reveals a well defined 2-fold symmetric four-helix bundle that projects ∼70 Å towards the cytoplasm. A second Fab, binding at a slightly different region of the C-terminus was used to determine the structure of the C-terminal domain of KcsA alone (Ser129 to Asn158) at 2.6 Å resolution. Binding of the Fab does not exert a major influence on KcsA single channel behavior, suggesting that the Fab epitope in the C-terminus does not undergo major conformational changes upon gating. Superposition of full-length and truncated KcsA main chains shows that the C-terminal deletion promotes a $\sim 15^{\circ}$ bending away from central axis of symmetry (residues Ala108 to His124), that the narrowest point along the inner bundle gate moves from residue Ala108 to V115 and that it contracts from ∼3.5 Å to < 2.5 Å in diameter. We suggest that this structure constitutes a better representation of the physiologically-relevant closed conformation of KcsA.

Platform X: Membrane Transporters & Exchangers

945-Plat Single-molecule FRET Study of Conformational Dynamics in Reconstituted LacY

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The N- and C-terminal six-helix bundles of lactose permease (LacY) form a large internal cavity open on the cytoplasmic side and closed on the periplasmic side with a single sugar-binding site at the apex of the cavity near the middle of the molecule. During sugar/H+ symport, an outward-facing cavity is thought to open with closing of the inward-facing cavity so that the sugar-binding site is alternately accessible to either face of the membrane. We use single-molecule fluorescence (Förster) resonance energy transfer (smFRET) to test this model with wild-type LacY and a conformationally restricted mutant. Pairs of Cys residues at the ends of two helices on the cytoplasmic or periplasmic sides of wild-type LacY and the mutant were labeled with appropriate donor and acceptor fluorophores, single-molecule fluorescence resonance energy transfer was determined in the absence and presence of sugar, and distance changes were calculated. SmFRET studies of LacY in detergent micelles revealed sugar-dependent conformational changes consistent with the alternating access model. However, upon reconstitution into liposomes, large distance changes in the molecule are detected even in the absence of sugar in diffusing liposomes; efforts are in progress to follow such dynamics on single, surface-tethered liposomes. The observed distance changes may reflect the effect of a true membrane environment on the dynamics of membrane proteins.

946-Plat Homodimer of the Mitochondrial Phosphate Transport Protein (PTP). In vitro Formation

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The concept of homodimeric mitochondrial transport proteins was first established with hydrodynamic studies of purified proteins. These elegant studies were difficult to carry out and quantitate due to the presence of large amounts of the detergent Triton X-100. Nevertheless the conclusion was reached that the ADP/ATP translocase, purified in a reconstitutively active form is a homodimer. Similarly, inhibitor titration studies of the ADP/ATP translocase, also somewhat difficult to analyze rigorously, lead to the conclusion that only one inhibitor molecule per two subunits of ADP/ATP carrier are required to block transport. To identify a homodimeric structure of mitochondrial transporters with transport function (1) we constructed two types of phosphate transport protein subunits that differed only in their affinity tag (His tag, FLAG tag). Such constructs permit the generation of PTP dimers with only one of each of these subunits. Reacting one of these subunit with N-ethylmaleimide (an inhibitor of phosphate transport) and combining it with the other subunit yields, as expected from a functional dimer, an inactive transporter. We have now characterized more carefully the interaction between PTP subunits, i.e. a wild type subunit and a subunit with a His tag bound to a Talon affinity column. We have demonstrated that homodimers do readily form and that the affinity between the subunits is significantly decreased when Cys28 is replaced with an Asp. This decrease in affinity between the subunits, predicted from disulfide formation results (2), is most likely due to both steric and charge repulsion effects.

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947-Plat Structure And Elastic Properties Of Tunneling Nanotubes

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Tunneling nanotubes (TNT's) are recently reported interconnection channels among cells, assumed to represent a novel mechanism for cell-cell interactions. They mediate actin-based transfer of vesicles and organelles and they allow signal transmission between cells. We describe, to our knowledge for the first time, the effects of lateral pulling with polystyrene beads trapped by optical tweezers on TNT's linking separate U87 MG human glioblastoma cells in culture. This cell line was chosen for handling ease and possible pathology implications of TNT persistence in communication

between cancerous cells. Observed nanotubes are shown to have the characteristic features of TNT's. Optical tweezer forces are exerted laterally on TNT's through the attached polystyrene beads and the results are followed by videomicroscopy, with simultaneous measurement of the exerted forces. We find that pulling induces two different types of TNT bifurcations. In one of them, termed V-Y bifurcation, the TNT is first distorted into a V-shaped form, following which a new branch emerges from the apex. In the other one, termed I-D bifurcation, the pulled TNT is bent into a curved arc of increasingly broader span. Curves showing the variation of pulling force with displacement in both situations are obtained. Results yield information on TNT structure, which appears to be analogous to that of thin filopodia, and order-of-magnitude estimates of TNT elastic properties (membrane bending rigidity and surface tension).

948-Plat Structural Investigations of the Cell-Penetrating Peptide SAP in Lipid Membranes

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Cell-penetrating peptides (CPPs) are a diverse group of polypeptide sequences capable of transporting various cargoes into cells without interfering with vital cell functions. The molecular mechanism(s) governing membrane transduction, an obligatory step of cargo delivery into the cytosol, are controversely discussed. One of the novel designer-made CPPs with very low cytotoxicity is the "Sweet Arrow Peptide" [SAP, $(VRLPPP)_3$], which adopts a stable amphiphilic polyproline II (PPII) conformation in aqueous solution due to its 50% Pro content in the primary sequence. SAP was demonstrated to self-assemble into regular fibrils preserving the PPII structure, and it can translocate covalently attached fluorescent dyes more efficiently than other established CPPs. Its unusual conformation and tendency to oligomerize appear to be relevant for its functional mechanism upon interacting with lipid membranes. To investigate its structure in membrane-mimicking environments we have synthesized several ^{19}F-labeled SAP analogues for solid state NMR studies. By CD we found a preference of the peptide in binding to neutral over negatively charged membranes. By oriented CD measurements and ^{19}F-NMR in oriented membrane samples, we could demonstrate that SAP assumes predominantly PPII conformation in liquid crystalline membranes, and that it aligns surfacially on the bilayer surface irrespective of concentration. Monitoring the mobility of the membrane-bound peptide, no evidence for the formation of extended self-assemblies was observed. However, several distinct conformational states seem to coexist with PPII under such conditions. In gel-state lipid bilayers, the peptide reversibly undergoes re-alignment, loses its mobility, and presumably changes its conformation. We have also analysed the conformational states of membrane-bound SAP using MD simulaions with orientational constrains from ^{19}F-NMR. The role of the observed structural features of SAP in the functionally relevant peptide-membrane interactions will be discussed.

949-Plat Molecular mechanism of phospholipid transfer by lipid transporter protein Sec14p

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>Sec14p is a major yeast phosphatidylinositol (PI)/phosphatidylcholine (PC) transfer protein that promotes the energy-independent transfer of either PI or PC between lipid bilayers in vitro. Although crystal structure of Sec14p is available, the detailed mechanism of lipid binding remains to be evaluated. Here we report on multifrequency electron paramagnetic resonance experiments to analyze dynamics as well as the electrostatic and hydrogen bonding microenvironment for series of *doxyl*-labeled PC molecules bound by Sec14p in a soluble protein:PC complex.[1] Partially resolved 130 GHz EPR spectra from *n-doxyl*-PC molecule bound to Sec14p were assigned to a hydrogen-bonded and a non-hydrogen bonded nitroxide species. Analyses allowed us to calculate the fraction of hydrogen-bonded nitroxide species and to characterize polarity and proticity profile along the phospholipid-binding cavity of Sec14p.[2] The data suggest that water molecules are drugged into the protein cavity upon the lipid binding. Proposed lipid exchange mechanism indicates that the polarity gradient inside Sec14p cavity contributes to the driving thermodynamic force for extracting a single phospholipid molecule from the bilayer. Proposed mechanism is being confirmed by X-ray crystal structure.

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950-Plat Interactions Between Phosphatidylethanolamine Headgroup And A Multidrug Transporter: A Conserved Mechanism For Proton Gradient Sensing?

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In a number of cases, the function of membrane proteins appears to require the presence of specific lipid species in the bilayer. We have shown that the secondary multidrug transporter LmrP requires the presence of phosphatidylethanolamine (PE), as its replacement by phosphatidylcholine (PC) inhibits transport activity and directly affects its structure, though the underlying mechanism was unknown (Gbaguidi, B., Hakizimana, P., Vandenbussche, G., and Ruysschaert, J. M. (2007) Cell Mol. Life Sci. *64*, 1571–1582). Here, we show that the effect of PE on the structure and the function of LmrP is mediated by specific interactions between the lipid headgroup and the protein. We used methyl-PE and dimethyl-PE analogs of PE to show that only replacement of the three hydrogens

by methyl moities leads to changes in the structural and properties of the reconstituted protein. This clearly indicates that LmrP does not depend on the bulk properties of the phospholipids tested but solely on the availability of one or more protons on the headgroup. We then show that a single point mutation in LmrP, D68C, is sufficient to recapitulates precisely every biochemical and biophysical effect observed when PE is replaced by PC, including energy transfer between the protein tryptophans and the lipid headgroups. We conclude that the negatively charged D68 is most likely involved in the interaction between LmrP and PE, and that such interaction is required for proton gradient sensing, substrate binding and transport. As D68 belongs to a highly conserved motif in the Major Facilitator Superfamily (which includes LacY, EmrD), this interaction might be a general feature of these transporters, and is involved in proton gradient sensing and lipid dependence.

951-Plat Solid State Nmr And Biophysical Studies On Multidrug Efflux Pumps From The Smr Family

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Transport proteins exhibiting broad substrate specificities are major determinants for the phenomenon of multidrug resistance. One of the key problems is to understand drug recognition in the context of their structural diversity. We have used 1H MAS NOESY NMR to screen the membrane interaction of many different molecules and found a similar interaction pattern (Siarheyeva et al. 2006). This supports the hypothesis that the membrane might act as a potential selectivity filter. Efflux pumps of the small multidrug resistance family bind antibiotics and transport them across the membrane in exchange for protons. The transport cycle must involve various conformational states of the protein needed for substrate binding, translocation and release. We show the existence of an occluded substrate-transporter complex for the EmrE homologue M.tuberculosis TBsmr and its substrate ethidium bromide by fluorescence spectroscopy (Basting et al. 2007). The pH gradient needed for antiport has been generated by co-reconstituting TBsmr with bacteriorhodopsin. Our findings support a model with a single occluded intermediate state in which the substrate is highly immobile. SMR transporters are functional dimers. We have used double quantum filtered 13C MAS NMR to probe the dimerisation interface of the E.coli multidrug transported EmrE. Essential residues were selectively labelled using cell free expression. Chemical shift and line shape analysis did reveal the formation of an asymmetric homo dimer.

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952-Plat Dynamic Imaging The Cytosolic Ph Of Individual Phagocytosing Human Neutrophils By Shifted Excitation And Emission Ratioing Of Fluorescence (seer)

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Phagocytosis is the internalization of microbes by phagocytes in order to kill and digest them. Phagocytosis is accompanied by activation of the NADPH oxidase complex that transfers electrons into the phagosome to produce superoxide anion, leaving protons in the cytosol. Here we employ a highly sensitive confocal microscopy technique to examine the cytosolic pH (pH_i) of individual human neutrophils during phagocytosis.

Human neutrophils were allowed to adhere to glass coverslips, incubated with 10 μM 5-(and 6)-Carboxy SNARF-1 in HBSS for 30 min at 37°C, and washed. SEER imaging (Launikonis et al., 2005, *J. Physiol. 567*: 523) was performed by simultaneously acquiring two confocal images: F_1, Excited at 514 nm and Emitted at 500–604 nm and F_2 (Ex at 594 nm and Em at 620–715 nm). F_1/F_2 monitors $[H^+]$ with a dynamic range of ∼150. Experiments were performed at room temperature. Resting neutrophils had a pH_i of 7.03 ± 0.03 (mean ± SEM, $n = 35$). Upon the initial engulfment of an OPZ particle, pH_i decreased rapidly for ∼5 min, with a maximum rate of −0.13 ± 0.01 pH units/min ($n = 18$), reaching a minimum pH_i of 6.49 ± 0.17 ($n = 18$). Non-phagocytosing cells did not acidify. Acidification of phagocytosing cells was prevented by 20 μM DPI, implicating NADPH oxidase as the source of the protons. When incubated with 100 μM Zn^{2+} resting pH_i was 6.95 ± 0.05 ($n = 9$), the maximum rate of acidification of phagocytosing cells was −0.37 ± 0.04 units/min, and the minimum was 5.9 ± 0.04 ($n = 9$). Thus, activation of NADPH oxidase in phagocytosing neutrophils produces rapid cytosolic acidification that is limited by proton current.

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Platform Y: Muscle Regulatory Proteins

953-Plat A Troponin Chimera To Study Troponin Dynamics And Interactions

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The troponin complex is responsible for the regulation of muscle contraction, and is composed of three subunits: troponin C, troponin I and troponin T. Troponin T is responsible for transmitting the conformational changes to the rest of the muscle thin filament, troponin I inhibits the interaction between actin and myosin, and troponin C is a Ca^{2+}-dependent switch which interacts with troponin I, removing the inhibition in the presence of calcium. While the atomic structure of most of the core region of the troponin complex has been determined by X-ray crystallography (Takeda et al., *Nature* ***424***, 35; Vinogradova et al., *Proc. Natl. Acad. Sci. USA*