the ion-conducting open state, rapid structural exchange between two conformations, presumably K+ high- and low-affinity, of the filter is observed. These millisecond dynamics provide the basis for simultaneous ion selection and gating of the selectivity filter at a timescale distinguishable from that of the gating action by the TM2.

58-Symp The human voltage-dependent anion channel

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The human voltage-dependent anion channel (VDAC) is a 283-residue integral membrane protein located in the outer mitochondrial membrane. It forms an aqueous pore through which metabolites and other small molecules pass between the cytosol and the inter-membrane space. The essential life-sustaining function of VDAC in metabolite trafficking is regulated by proteins of the Bcl-2 family, and previously pro- and anti-apoptotic Bcl-2 proteins have been shown to interact with VDAC.

We have expressed, purified, and refolded VDAC1 into LDAO detergent micelles. We have shown that this preparation is amenable to NMR studies exhibiting excellent multidimensional spectra. The recombinant protein is functional as it is capable of interacting with substrates and inhibitors. A large fraction of the backbone and side-chain resonances were assigned and secondary structure elements were identified through analysis of chemical shifts and inter-strand Nuclear Overhauser effects (NOEs). Initial models of the polypeptide fold were compared with predicted models of the channel. Determination of the tertiary structure is in progress. Various biophysical experiments indicate that micelle-bound VDAC is in intermediate exchange between monomer and trimer. Using NMR spectroscopy, gel filtration, and chemical cross-linking we obtained direct evidence for binding of Bcl-xL to VDAC in a detergent micelle system. The VDAC-interacting region of Bcl-xL was characterized by NMR with chemical shift perturbation and transferred cross saturation. The interaction region was mapped to a putative helical hairpin motif of Bcl-xL that was found to insert into detergent micelles. Our results suggest that Bcl-xL can bind to 1 or 2 VDAC molecules forming heterodimers and heterotrimers. Our characterization of the VDAC/Bcl-xL complex offers initial structural insight into the role of anti-apoptotic Bcl-xL in regulating apoptotic events in the mitochondrial outer membrane.

Symposium 4: Putting the Move on Myosin

59-Symp Molecular Movements Associated with Force Generation

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The myosin actin-binding cleft located between the upper and lower 50kD domains is thought to undergo changes as a function of nucleotide state and binding to actin. Cleft closure is needed to allow tight binding to actin and might be coupled to the opening of active site. To detect cleft closure we measured the distances between the upper- and lower-domains with various nucleotides and in the presence of actin using conventional and pulsed dipolar EPR (DEER). Double cysteine mutants were engineered in upper domain and in lower domain that were labeled with spin probes/fluorophores. We found two major populations of distances and shifting in the populations as a function of nucleotide state and actin binding. The average distances and the trends in distance distribution were in excellent agreement between FRET and EPR. In presence of actin, the short distance population increased significantly and broad distance distribution was found, which suggest that the cleft is closed upon actin binding, however, multiple conformations exist. In smooth muscle, force generation is regulated by phosphorylation of regulatory light chain (RLC). The molecular mechanism is uknown, although it is clear that it involves the interaction between the myosin heads. To determine the relative positions of the heads we have measured distances between the selected cysteine mutants of RLC exchanged into smooth muscle myosin. In the unphosporylated SMM monomers, the measured distances were 11.5 Å for C38, 28 Å for C59 and over 40 Å for C108 and C84. Upon phosphorylation of SMM all the distances were beyond the sensitivity range of EPR. Using novel distance geometry algorithm, we have a constructed a model for the 6S monomer. Our model at this stage is not unique but the family of current solutions includes model of Wendt & Taylor.

60-Symp Structural Studies Of Myosin VI Provide Important Insights For The Motor Mechanism Of Myosin Motors

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Force is produced by myosin motors upon the actin-driven conformational changes of the motor that leads to sequential release of the hydrolysis products of ATP. Structural information on the multiple states the motor adopt along its motor cycle is essential for understanding how chemical energy is converted into force production. Myosin VI is the most enigmatic of myosins. First, it produces its force and traffics toward the minus-end of actin filament in the opposite direction to other myosins. Second, this motor uses a number of unique mechanisms that are not well understood to take multiple steps on an actin filament without detachment. Surprisingly, these steps are similar in size to those of myosin V, even though the lever arm of myosin VI contains only one IQ motif, whereas that of myosin V contains six. We have recently revealed the structure of the myosin VI motor at the end of its powerstroke (in the rigor conformation). This structure reveals that a specific insert wraps around the converter and binds a calmodulin that interacts with the converter. The result is a ~120° repositioning of the myosin VI lever arm, which explains its reverse directionality. However, to account for the large powerstroke of myosin VI, this study clearly predicted that the pre-powerstroke state of myosin VI must differ from that of plus-end directed myosins. We have recently solved this structure. It reveals that unexpected rearrangements in the converter are critical to position the lever arm ideally to produce a very large stroke.

61-Symp The Molecular Mechanism of the Activation of Myosin Filaments

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Muscle contraction involves interaction of myosin heads of thick filaments with actin thin filaments. Myosin-linked regulation can occur by phosphorylation of the myosin regulatory light chains (RLC). EM of 2D-crystal vertebrate smooth muscle myosin provided insight into the relaxed (switched-OFF) myosin structure, achieved by asymmetric intra-molecular interactions between both heads, switching them OFF. Cryo-EM revealed that this interacting-heads structure is present in thick filament of tarantula striated muscle. We calculated an IHRSR reconstruction of frozen-hydrated tarantula thick filaments including the filament tilt, which increased the yield of images used, reaching 2nm resolution. The reconstruction reveals new densities: one between the free-head and its S2, and two between the free-head and the neighbor blocked-head. To interpret these intermolecular interactions, we sequenced the tarantula RLC. Using SITUS we fitted an interacting-heads atomic model that included the tarantula RLC homology model, its 52aa N-terminal fragment predicted model and the human cardiac S2 crystal structure. The fitting revealed an intramolecular interaction between the cardiomyopathy loop (Arg-411) of the free-head and its S2 and two intermolecular interactions between the cardio-loop (Arg-371) and the 297–326 loop of the free-head with the N-terminal fragments of the blocked-head essential and regulatory light chains. These intermolecular interactions help to establish the thick filament switched-OFF relaxed state. Phoshorylation of Ser-45 of the RLC N-terminal fragment could weaken this intermolecular interaction, as suggested by secondary structure predictions; helping to release both heads, activating the thick filament.

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62-Symp Navigating the Cytoskeleton with Myosin X

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Eukaryotic cells organize their contents through trafficking along cytoskeletal filaments. The leading edge of a typical metazoan cytoskeleton consists of a dense and complex arrangement of cortical actin. A dendritic mesh is found across the broad lamellopodium, with long parallel bundles at microspikes and filopodia. It is currently unclear if and how myosin motors identify the few actin filaments that lead to the correct destination, when presented with many similar alternatives within the cortex. Here we show that myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. Myosin X moves individual actin filaments poorly in vitro, often supercoiling actin into plectonemes. However, single myosin X motors move robustly and processively along fascin-actin bundles. This selection requires only parallel, closely spaced filaments, as myosin X is also processive on artificial actin bundles formed by molecular crowding. Myosin X filopodial localization is perturbed in fascin-depleted HeLa cells, demonstrating that fascin bundles also direct motility in vivo. Our results demonstrate that myosin X recognizes the local structural arrangement of filaments in long bundles, providing a mechanism for sorting cargo to distant target sites.

Minisymposium 1: Structure-Function of Oxidative Pathway Proteins

63-Minisymp Building a Function into Simplified Structures: Membrane Spanning Maquettes with Chains of Redox Cofactors

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Heme proteins play a pivotal role not only in energy generation but also in controlling cell life and death. Many of the key functions are accomplished through complicated system of enzymes and redox carrier molecules that control electron and proton transport. Even though significant number of these enzymes has been structurally characterized, the actual mechanism of redox catalysis is not always understood. Therefore we have adopted a different approach to address the structure-function of redox proteins: we aim to uncover the assembly instructions of function in proteins using smaller, simpler, more robust model proteins, maquettes. Our questions ask how many engineering elements are required to achieve a particular biological function, what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function. To start answering these questions, we have synthesized a set of amphiphilic maquettes (AP6 series). The 44 amino acid long, tetrameric AP-6 maquettes assemble with up to six ferric hemes b per tetramer in three different positions (two hemes per position). We will report how these maquettes transfer electrons across membranes, bind O2 and CO. These AP6 maquettes also co-solubilize with diblock copolymers or lipids and on an air-water interface, they compress to specific surface pressure, and can be transferred by Langmuir - Blodgett technique on HOPG (highly ordered pyrolytic graphite) surface for single molecule measurements of electrical properties by scanning probe microscopy.

64-Minisymp Structure, Function and Evolution of Reaction Center Bound Cytochrome c

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