association and crowdinginduced steric repulsions is sufficient to explain both the size and dynamics of syntaxin clusters and likely of many oligomerizing membrane proteins that form supramolecular structures.

2646-Plat Synchrotron X-ray Diffraction Study of Neurofilament Networks Interaction under Osmotic Pressure

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Neurofilaments (NFs), a major constituent of nerve cell axons, assemble from three subunit proteins of low, medium and high molecular weight to form a 10 nm diameter rod with radiating unstructured sidearms. The sidearm interactions result in NF physical hydrogel with the NF long axis running parallel to the axon. The NF network imparts mechanical stability to the axon and acts as a scaffold for microtubules. Here, we reassemble NFs in vitro from varying ratios of the subunit proteins, purified from bovine spinal cord. At high protein concentration, the NFs form a nematic hydrogel network with a well-defined interfilament spacing as measured by synchrotron small angle x-ray scattering (SAXS). The phase diagram demonstrating the transition between the nematic and isotropic gel phases in relation to varying side-arm grafting density has been mapped out [1]. Using the SAXS-osmotic pressure technique reported previously for microtubules [2] we directly probe the polyampholyte brush interactions between NF sidearms. We present data on the interfilament spacing at different osmotic pressure and at different salt and sidearm concentrations which begin to reveal the non-trivial electrostatic nature of the interfilament interactions within the NF hydrogel.

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2647-Plat Structure of the Plexin-B1 Effector Domain in Complex with the small GTPase Rac1 derived from NMR restraints: Characterization of the interaction surface by NMR, molecular modeling and thermodynamic measurements

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Plexin-B1 functions in axon guidance and cell motility. It is the first transmembrane receptor that directly binds to small GTPases. We recently determined the NMR solution structure of a monomeric form of the Rho GTPase binding domain (RBD) of human plexin-B1. Remarkably, three different Rho family GTPases, Rac1, Rnd1 and RhoD, bind to this central cytoplasmic region, which was shown to adopt a ubiquitin-like fold. The same surface of this structure is involved in the interaction with the three GTPases as shown by Nuclear Magnetic Resonance (NMR) surface mapping experiments, suggesting a common role for this domain as a GTPase effector. In order to determine the details of the interaction, NMR restraint-driven docking was used to calculate the structure of the Rac1 - RBD plexin-B1 complex employing Haddock2.0. It should be noted RBD was target 31 - the first NMR target - in a recent CAPRI challenge aimed at a blind prediction of the complex structure. Molecular modeling is used to suggest differences between GTPases that bind to the plexin-B1 RBD (Rac1, Rnd1 and RhoD) and those that do not interact (incl. Cdc42 and RhoA). The results are compared to mutagenesis data on the GTPases in a thermodynamic analysis to confirm our understanding of the protein-protein interaction surface and its specificity. The complex with Rac1 also shows that the GTPase binding site partially overlaps with a receptor dimerization interface and suggests a mechanism for destabilization of a dimeric receptor by GTPase binding.

Symposium 20: ABC Transporters: Molecular Structures and Mechanisms

2648-Symp Maltose Transporter Cycle

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The maltose transporter from E. coli is an ATP-binding cassette transporter that mediates the uptake of maltose and longer chain maltodextrins. After crossing the outer membrane, maltodextrins are bound with high affinity by a periplasmic maltose binding protein MalE and delivered to a membrane-bound complex of transmembrane membrane proteins MalF and MalG and two copies of an ATPase, MalK. If ATP hydrolysis is blocked by mutation of a conserved glutamate following the Walker B motif of MalK, addition of ATP to the transporter traps a high affinity complex MalEFGK2 that represents a catalytic intermediate in the transporter cycle. Both the biochemical analysis and the high resolution structure of this intermediate are consistent with a concerted mechanism for transport in which the interaction between maltose-bound MalE and ATP-bound MalFGK2 triggers a global conformational change that results in simultaneous activation of ATP hydrolysis and transfer of maltose from MalE to a sugar binding site lying deep within a cleft between MalF and MalG. The high resolution structure of this EFGK2 intermediate will be discussed, together with the results of biochemical analyses that suggest the structure represents an intermediate of the translocation cycle.

2649-Symp News on ABC and CBS

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Cystathionine-β-synthase (CBS) domains are found in over 10,000 proteins in species from all kingdoms of life, yet their functions are largely unknown. Tandem CBS domains are associated with membrane transport proteins, most notably members of the ATP-binding cassette (ABC) superfamily, voltage-gated chloride channels and transporters, cation efflux systems, and various enzymes, transcription factors, and proteins of unknown function[1]. We have shows that tandem CBS domains in the osmoregulatory ABC transporter OpuA are sensors for ionic strength that control the transport activity through an electrostatic switching mechanism. The on/off state of the transporter is determined by the surface charge of the membrane and the internal ionic strength which is sensed by the CBS domains[1,2]. By engineering of the CBS domains, we have been able to construct OpuA molecules that are no longer salt-dependent but also transporters with an increased ionic threshold for activation[2,3]. Recent progress on the functional and structural analysis of the ABC transporter OpuA and the regulatory role of the CBS domains will be presented.

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2650-Symp Structural Dyanmics of the ABC Transporter MsbA

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ABC transporters harness the free energy of ATP hydrolysis to power the thermodynamically unfavorable trafficking of a wide spectrum of substrates in and out of the cell. In Gram-negative bacteria, the transport of lipid A from its site of synthesis across the inner membrane is critically dependent on the expression of the ABC transporter MsbA. Orthologs of MsbA, including human P-glycoprotein, bind and transport cytotoxic molecules and are associated with multidrug resistance phenotypes. We used site-directed spin labeling and EPR spectroscopy to determine the conformational motion that couple energy expenditure to substrate translocation in MsbA. Spin labels were systematically introduced along 5 transmembrane helices and the adjacent extracellular and intracellular regions and at selected locations in the nucleotide binding domains. Distances between symmetry related spin labels in the dimer were used to monitor the relative separation of the transmembrane domains, the dimer interface, and the packing of the NBDs during the ATPase cycle. Spin labels accessibility to paramagnetic reagents reported on water accessibility in the putative substrate binding chamber. We find that ATP hydrolysis fuels a relative motion of the NBDs close to 30 Å. The movement of the NBDs is coupled to reorientation of the substrate binding chamber from cytoplasmic-facing to extracellular-facing through large amplitude motion on either sides of the transporters. The closing of the chamber on the intracellular side leads to a significant restriction in NiEDDA and by inference water accessibility to the substrate binding chamber. These results are not consistent with current models deduced from studies of substrate-specific ABC importers that envision the two NBD in contact throughout the ATP hydrolysis cycle.

Symposium 21: Nucleic Acid-based Motors

2651-Symp Single Molecule Studies of Transcriptional Termination Reveal a Mechanism

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Transcription termination by prokaryotic RNA polymerase occurs at sequences that code for a G:C-rich hairpin in the RNA immediately followed by a 9-nt, U-rich tract. We used single-molecule optical trapping techniques to investigate the mechanism by which elements from three representative terminators (his, t500, and tR2) destabilize the transcription elongation complex. In wild-type (WT) terminators, loads exerted via the DNA did not affect the termination efficiency. However, the force-dependent kinetics for the release of transcripts carrying the t500 terminator sequence and the force-dependent termination efficiency of a t500 mutant imply a forward translocation mechanism for this particular terminator. Tension applied to isolated U-tract sequences (minus hairpins) induced transcript release in a manner consistent with a mechanism involving shearing of the RNA:DNA hybrid within the enzyme. We deduce that closure of the final 2–3bp at the base of the hairpin stem supplies energy sufficient to destabilize the hybrid, and we propose a quantitative model for termination based on both hairpin and hybrid energetics. The model successfully predicts the termination efficiency over a wide range of values, and also how this quantity varies with load for all three WT and several mutant terminators.