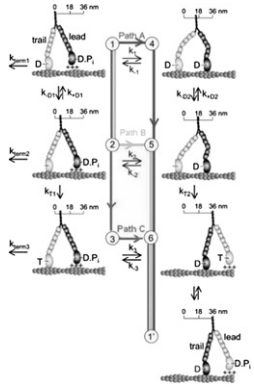
ATPase cycle (see Figure). The reduced run lengths with increasing ATP, ADP, and Pi suggest that runs terminate from two distinct states; one with both heads weakly-bound (state 3) another with ADP in the trailing head while the leading head has yet to undergo its powerstroke (state 1). In addition, to strain dependent accelerated ADP-release from the trailing head (State 4), the model also predicts that strain accelerates ATP binding (state 5) two-fold. These data and model analysis suggest that myosin Va processivity involves a complex branched kinetic pathway, providing the motor versatility when meeting the physical challenges presented by the intracellular environment.



2808-Plat

Bidirectional Cooperative Motion Of Myosin-II Motors On Actin Tracks With Randomly Alternating Polarities

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The cooperative action of many molecular motors is essential for dynamic processes such as cell motility and mitosis. This action can be studied by using motility assays in which the motion of cytoskeletal filaments over a surface coated with motor proteins is tracked. In previous studies of actin-myosin II systems, fast directional motion was observed, reflecting the tendency of myosin II motors to propagate unidirectionally along actin filaments. Here, we present a motility assay with actin bundles consisting of short filamentous segments with randomly alternating polarities. These actin tracks exhibit bidirectional motion with macroscopically large time intervals (of the order of several seconds) between direction reversals. Analysis of this bidirectional motion reveals that the characteristic reversal time, τ_{rev}, does not depend on the size of the moving bundle or on the number of motors, *N*. This observation contradicts previous theoretical calculations based on a two-state ratchet model (Badoual *et al.* 2002. *Proc. Natl. Acad. Sci.* 99:6696-6701), predicting an exponential increase of τ_{rev} with *N*. We present a modified version of this model that takes into account the elastic energy due to the stretching of the actin track by the myosin II motors. The new model yields a very good quantitative agreement with the experimental results.

Platform AU: Protein-Ligand Interactions

2809-Plat

The Lysine At Position 13 Of Pten'S N-terminus Is Necessary For Its Preferred Interaction With Pi(4,5)p_2

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Phosphatase and tensin homologue deleted on chromosome 10, also known as PTEN, has been identified as the most important regulator of the PI3K pathway, mutation or deletion of one copy of this protein results in a tumorigenic state. PTEN has also been identified as the second most important and mutated tumor suppressor, rivaled only by p53. PTEN contains within its N-terminus a $PI(4,5)P_2$ binding domain which has been shown to bind preferentially to $PI(4,5)P_2$, and whose presence within the protein is necessary for binding and activity of the enzyme. Within the $PI(4,5)P_2$ binding domain resides a lysine which is frequently mutated in many types of cancer, one of the most important mutations being $PTEN_{K13E}$. Because this mutation results in a change in the overall charge of the $PI(4,5)P_2$ binding domain, we have studied the effects of not only this mutation on the interaction of PTEN and its N-terminally derived peptide with $PI(4,5)P_2$, but have also mutated this lysine to arginine to maintain the overall charge of the binding domain, as well as moving only the position of this lysine within the N-terminus. We have found that mutation of this lysine, even those that maintain charge and overall identity of the residues within the $PI(4,5)P_2$ binding domain result in decreased ability to bind to $PI(4,5)P_2$ containing membranes. Interestingly, the proteins which have mutations at this lysine also do not undergo any conformational changes upon interaction with membranes containing $PI(4,5)P_2$, in contrast what was observed for the wild type protein. The lack of binding of these mutated proteins and subsequent conformational changes give insight into the mechanism of these mutations in the development of a tumorigenic state.

2810-Plat

Structural and Biophysical Characterization of the GAF Domains from Phosphodiesterases 5 and 6

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Phosphodiesterases 5 and 6 control the intracellular levels of cGMP through hydrolysis. The catalytic domains of both proteins are regulated by allosteric binding of cGMP to the N-terminal GAF domain (GAF A) of a tandem pair. We present the atomically detailed structures of both cGMP-bound GAF A domains as determined by NMR (PDE5A) and x-ray crystallography (PDE6C). Each domain adopts a conserved overall fold with well-defined cGMP binding pockets. However, the nucleotide coordination is distinct with a series of altered binding contacts. Nucleotide binding specificity is provided in each by the orientation of an Asp/Asn residue that is within hydrogen bond distance of the guanine ring. In PDE5A, a D196A mutation disrupts cGMP binding and increases cAMP affinity causing an altered cAMP-bound structural conformation in constructs containing only GAF A. NMR studies reveal that both GAF domains undergo significant cGMP-dependent conformational changes. In PDE5A, GAF B stabilizes the highly dynamic multi-state apo GAF A domain, presumably via direct interaction. In contrast, cGMP-free GAF A from PDE6C is more defined and in a single ''open'' state with flexible elements. Biophysical characterization of the GAF domains by Circular Dichroism and Analytical Ultracentrifugation further underlines the difference between the two PDEs. The structural features of the GAF domains from PDE5 and PDE6 revealed here provide a basis for future investigations of the regulatory mechanism of both PDEs and the design of GAF-specific small molecule inhibitors of PDE function.

2811-Plat

Mechanism Of Interaction Between The Volatile Anesthetic Halothane And A Model Ion Channel Protein: Fluorescence And Infrared Spectroscopy Employing A Cyano-phenylalanine Probe And Molecular Dynamics Simulation

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We demonstrate that cyano-phenylalanine (Phe_{CN}) can be utilized to probe the binding of the inhalational anesthetic halothane to a model ion channel protein hbAP-Phe_{CN} possessing a designed binding cavity. The Trp to Phe_{CN} mutation adjacent the cavity alters neither the α-helical conformation nor the 4-helix bundle structure. The halothane binding properties of hbAP-Phe_{CN}, based on fluorescence quenching, are consistent with those of the Trp-prototype, hbAP1. The dependence of fluorescence lifetime on halothane concentration implies a one-dimensional diffusion of halothane along the nonpolar core of the protein bundle. Consequently, the fluorescence quenching is dynamic at lower halothane concentrations, becoming static at higher concentrations. The 4-helix bundle structure present in aqueous detergent solution and at the air-water interface, is preserved in multilayer films of hbAP-Phe_{CN}, enabling vibrational spectroscopy of both the protein backbone and its nitrile label (-CN). The -CN stretching vibration exhibits a largely reversible blue-shift upon halothane binding.

The complexity of this 4-helix bundle protein, where four Phe_{CN} probes are present adjacent to the designed binding site within each bundle, all contributing to the infrared absorption, requires molecular dynamics simulation to interpret the infrared results. Decomposition of the forces acting on the nitrile probes indicates that -CN's blue shift arises from the halothane induced changes in the probes' electrostatic protein environment averaged over the four probe oscillators. Although halothane remains localized within the binding cavity, it undergoes significant translational and rotational motion, modulated by the interaction of halothane's $-CF_3$ group with backbone hydrogen atoms of residues forming the cavity. This halothane-backbone interaction strongly outweighs the halothane-probe interaction, making -CN a good ''spectator'' probe of the halothane-protein interaction.

2812-Plat

Binding Kinetics of Two Hyperactive Antifreeze Proteins are Revealed by Using Novel Microfluidic Devices

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Antifreeze proteins (AFPs) are produced by some cold-adapted organisms and function against freezing by arresting the ice crystal growth and preventing ice recrystalization. The questions regarding the binding kinetics of antifreeze proteins to ice surfaces are still a matter of debate and experimental data evaluating

the theories are limited. We have developed novel microfluidic devices to understand and question whether the binding of AFPs to ice surfaces is irreversible or reversible. Single ice crystals (~20-100 μm) grown in AFP solution can be kept in this controlled environment in which one is able to adjust the concentration of the protein that is in the solution and the temperature of the cell itself. We have tested two different hyperactive AFPs from spruce budworm and *Tenebrio molitor*; both are beta-helical and have a good shape complementary to ice surfaces. We demonstrate AFPs which are attached to ice crystals keep protecting the crystal once the flow turned on and solution is exchanged with AFP free buffer solution. We followed crystals in this AFP free buffer solution for hours within a constant temperature gradient. Our observations show that there is neither growth nor melting of the crystal observed in this continuous flow of buffer solution, and there cannot be an exchange of AFPs between solution and ice surfaces as it was claimed by some in the literature. Based on fluorescence microcopy and microfluidic devices, we conclude that antifreeze proteins from spruce budworm (sbwAFP) and *Tenebrio molitor* (TmAFP) are adsorbed to ice surfaces irreversibly, and thus our observations are in line with adsorption-inhibition theory.

2813-Plat

Dynamic and Strucutral Effects of Ligand and Coregulator Binding on Estrogen Receptor Ligand Binding Domain Measured by Electron Paramagnetic Resonance

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The estrogen receptor (ER) is an important therapeutic target for the treatment and prevention of estrogen responsive forms of breast cancer. Despite the availability of several crystal structures for ER bound to either agonist or antagonist ligands, its molecular mechanism of action still remains unclear. The major structural difference between agonist and antagonist forms can be observed in the position of helix-12 (H12) C-terminus region. Here, we present the results of site directed spin labeling on the H12 region (543) and on the H11-H12 hinge region (530) to monitor the effect of ligands with different biological activity on the solution dynamic and structure of H12. We found that the hinge region is directly affected by allosteric binding of coregulators peptides in a ligand dependent fashion. We characterized the structural changes resulting from ligand/coregulator binding using DEER spectroscopy. Additionally the effects of ligand binding on H12 were directly observable with our 543 labeled ER. When taken together, these results substantially complete our current understanding of the interplay between ligand/coregulator binding and dynamic/structural changes that regulate ER's biological activity.

2814-Plat

Structural Changes And Binding Kinetics Of Fluoro-tryptophan Substituted HyHEL-10 scFv Monitored Using 19F-NMR, High Resolution Crystal Structures And SPR-Biacore Analysis

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High affinity and specificity are hallmarks of the observed association of an antibody with its antigen. These characteristics are governed by a combination of non-bonded interactions and shape complementarity at the binding interface. The flexibility of the CDR binding loops plays a key role in the ability of the antigen to adapt to the surface of the antigen. We explored these relationships using 19-F fluorine NMR by measuring chemical shifts and T2 relaxation parameters for the binding of HyHEL-10 scFv antibody to hen eggwhite lysozyme and epitope-specific mutants. By incorporating 5-fluorotryptophan into the scFv we had a total of 6 NMR sensitive probes in its structure. Individual replacement of those residues with phenylalanine allowed for assignment of each peak in the NMR spectrum so that changes in signal could be analyzed in a site-specific way. A residue-specific analysis is shown including the structural changes occurring during binding. Analysis of binding kinetics using SPR (Biacore) coupled with high resolution crystal structures for the complex showed an unexpected and interesting impact of the 5-fluorotryptophan incorporation on binding affinity. Together these results provide new insights into the underlying structural and dynamic characteristics for tight association and high specificity in biomolecular protein interactions.

2815-Plat

Kinetics and Thermodynamics of Antibody Binding to B-Type Natriuretic Peptide

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B-type natriuretic peptide (BNP) is a naturally secreted regulatory hormone that influences blood pressure and vascular water retention. The plasma BNP concentration is a clinically recognized biomarker for various cardiovascular diseases. Quantitative detection of BNP can be achieved in immunoassays using high-affinity monoclonal antibodies. Temperature dependence of the equilibrium binding constants and the kinetic rates were studied for anti-BNP mAbs 106.3 and 3-631 by means of fluorescence spectroscopy. Thermodynamic parameters including changes in the free energy, enthalpy and entropy measured at equilibrium are in a good agreement with the parameters calculated from kinetics data. The differences in thermodynamic parameters measured for the two antibodies under study support structural data obtained by NMR and X-ray crystallography.

2816-Plat

Allosteric Regulation Across a β-Sandwich Protein: How a Bacterial Adhesive Protein is Activated by Mechanical Force

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We have previously proposed that allosteric regulation causes some receptors to form catch bonds, which are strengthened rather than weakened by mechanical force. Here we describe a crystal structure of the catch-bond forming bacterial adhesive protein FimH in native fimbrial tips that is dramatically different that previous structures of FimH. The new structure shows how a neighboring domain allosterically inhibits the adhesive domain. In previous crystal structures, the adhesive domain was pre-activated by prevention of these native inter-domain contacts. Molecular dynamic simulations and structural analysis show how mechanical force breaks the native contacts between the autoinhibitory domain and the lectin domain, and how conformational changes in the interdomain region regulate the ligand-binding pocket. These structural changes explain how biochemical and mechanical stimuli affect binding in experiments. Together, these data provides the structural details for how FimH forms allosteric catch bonds. Surprisingly, the FimH adhesive domain has a beta-sandwich motif, a class considered to be structurally rigid. However, different parts of the sandwich can lever open like a pair of pliers, causing large correlated changes in both distal loop regions in spite of only small changes in the fulcrum at the center of the sheets.

Platform AV: Membrane Physical Chemistry II

2817-Plat

Polar Residues in Transmembrane Helices can Dramatically Reduce Mobility on SDS Gels WITHOUT Dimerization

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Polar residues in membrane-spanning helices are known to drive oligomerization in biological and synthetic membranes, as well as in membrane mimetic systems such as detergents. Here we report a study of a hydrophobic peptide that contains either an asparagine (N) or a leucine (L) residue in the 12th position of an otherwise hydrophobic segment of 20 amino acids. These peptides are fully alpha-helical in detergents. In SDS polyacrylamide gels, the L12 peptides migrated as monomers while the N12 peptides always migrated as single bands at twice their apparent molecular weights. In sharp contrast, Forster resonance energy transfer (FRET) experiments in SDS showed little evidence of dimerization of N12 under any conditions studied. Experiments were done with labeled peptides at concentrations up to 50 micromolar and at acceptor to donor ratios from 1:1 to 10:1. SDS concentrations ranged from 3.5 to 70 mM. We also performed in situ FRET experiments on the peptide bands in polyacrylamide gels, where there was little excess FRET observed for the slower N12 bands relative to L12 bands. We conclude that N12 is always monomeric in SDS gels, despite the fact that it appears to migrate as a dimer. Dynamic light scattering experiments showed a significant difference between L12/SDS micelles and N12/SDS micelles. We hypothesize that the polar residue in the center of the otherwise hydrophobic helix alters the interactions between the peptides and detergent and that physical differences in the peptide-detergent micelles, such as shape and stoichiometry are responsible for the altered migration of the N12 peptides relative to the L12 peptides.

2818-Plat

Examining The Role Of Lipid Variations And Proteins On Membrane Biophysics: Synthetic Versus Natural Membrane Vesicles

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Biomembranes in living cells are complex, heterogeneous and dynamic systems that regulate numerous biological processes such as cell signaling,