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,