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dynamics of γ in solution and iii) the interaction of γ with membranes. The externalization process has been investigated using steered MD simulations (SMD) under differing protonation states to mimic differing pH conditions. SMD simulations under acidic conditions exhibited structural transitions in the capsid distinct from neutral condition simulations. The structural dynamics of γ have been investigated using metadynamics simulations and indicate γ has a low barrier separating helical and disordered states. The influence of γ structure on membrane binding has been investigated using the MARTINI coarse-grained model to calculate binding free energies. These combined studies have provided new structural and thermodynamic insights into the post-entry stages of non-enveloped virus infection.

198-Plat
Reconstitution of Multivalent PDZ Domain Binding to the Scaffold Protein PSD-95 Reveals Ternary-Complex Specificity of Combinatorial Inhibition

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Multidomain scaffold proteins serve as hubs in the signal transduction network. By physically colocalizing sequential steps in a transduction pathway, scaffolds catalyze and direct incoming signals. Much is known about binary interactions with individual domains, but it is unknown whether ''scaffolding activity'' is predictable from pairwise affinities. Here, we characterized multivalent binding to PSD-95, a scaffold protein containing three PDZ domains connected in series by disordered linkers. We used single molecule fluorescence to watch soluble PSD-95 recruit diffusing proteins to a surface-attached receptor cytoplasmic domain. Different ternary complexes showed unique concentration dependence for scaffolding despite similar pairwise affinity. The concentration dependence of scaffolding activity was not predictable based on binary interactions. PSD-95 did not stabilize specific complexes, but rather increased the frequency of transient binding events. Our results suggest that PSD-95 maintains a loosely connected pleomorphic ensemble rather than forming a stereospecific complex containing all components.

199-Plat
Quantifying Protein-Protein Binding Energy and Entropy using Molecular Dynamics Simulations

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Protein-protein interactions (PPIs) represent a critical aspect of a wide range of biological processes, as most proteins need to associate to various binding partners to carry out their functions. While the basic principles of PPIs are generally understood, it is a grand challenge to accurately predict the binding affinity quantitatively for any given complex. Recently, this potential of mean force (PMF)-based methodology was extended to a much larger protein-peptide system, and we accurately determined the absolute binding free energy between the receptor protein and the ligand peptide. We plan to employ our PMF-based methodology to probe effects of mutations in thermodynamics of protein-protein interaction. Furthermore, thermodynamic decomposition of barstar-barnase protein complex association is attempted to quantify not only to probe the effect of mutation on binding free energy but also on binding entropy.

200-Plat
Generation of Electrochemical Gradient from Peptide Self-Assembly

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The nucleating core of the Amyloid β peptide associated with Alzheimer's disease is able to organize into a peptide bilayer maintaining dimensions similar to biological phospholipid membranes. However, the dynamics of the hydrogen-bonded peptides are certainly different from the flexible alkanes of the lipid membranes, and this crystallinity prompted us to consider their dynamics and functional potential. Here we will review these unique surfaces built on cross-β structures of amyloid, and extend that pattern to mixing of positively- and negatively-charged surfaces to give even higher order architectures with high electrochemical potential across the 4 nm membrane. Further, we have developed EFM analyses to map the charge distribution and explored novel energy or electron transduction reactions. Our results show that these peptide membrane scaffolds are dynamic, and their ability to self-organize offers a new opportunity for engineering specific molecular recognition elements into the peptide assemblies and well-ordered materials.

Platform: Member Organized Session: Protein Nanoassemblies and Networks in Bacterial Chemotaxis and other Two-component Signaling Systems

201-Plat
Insights from Phosphorylation Profiling of an Autoregulated Two-Component System

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Connecting biophysical and biochemical parameters determined *in vitro* to understanding and predicting physiological behavior is an important challenge. Bacterial two-component systems have long served as models for investigating fundamental properties of signal transduction. While understanding regulatory mechanisms has benefited greatly from mathematic modeling, a major obstacle to this approach is the lack of quantitative analyses of two-component systems in their native environments, particularly *in vivo* parameters for histidine kinase and phosphatase activities. Measurement of cellular phosphorylation levels combined with mathematic modeling has enabled a phosphorylation profiling approach to investigate whether protein expression levels of the archetype PhoB/PhoR two-component system are optimized to the phosphorylation output profile and how the positive autoregulatory scheme enables wild-type cells to achieve optimal expression levels of PhoB/PhoR in dynamic environments. The PhoB/PhoR system responds to phosphate (Pi) limitation, and different Pi conditions were discovered to have conflicting requirements for optimal protein expression levels. Experimental evidence established that wild-type cells achieve different optimal expression levels via autoregulation under respective Pi conditions. The fitness optimum balances costs of protein production with benefits, which are correlated with the phosphorylation output. Laboratory evolution experiments revealed that cells with different non-optimal levels of PhoB/PhoR all rapidly evolve toward optimal expression levels by acquisition of diverse mutations, demonstrating strong selective pressure for evolutionary tuning of protein expression levels. However, positive autoregulation comes at the cost of delaying the output response. Analysis of promoter architecture and mathematic modeling suggests that a PhoB repressor site within the *phoBR* promoter provides negative feedback that enables acceleration of the response, counterbalancing the delay imposed by positive autoregulation. Thus system architecture appears to be exquisitely evolved to provide protein activities, levels and timing of expression integrated for optimal response output under dynamic and diverse conditions.

202-Plat
Hydrogen Exchange Reveals Differences between Bacterial Chemoreceptor Signaling States

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Bacterial chemotaxis receptors form membrane-bound nanoarrays that sense and adapt to ligands from the environment. These arrays consist of receptors interacting at their cytoplasmic tips with a ''baseplate'' formed by the CheA kinase and CheW coupling protein, ≈300 Å away from the periplasmic ligand binding sites. Receptor methylation at 4 glutamate residues in the cytoplasmic domain mediates adaptation to ongoing stimuli. To determine what ligand- and methylation-induced changes in the receptor cytoplasmic domain control the kinase activity, we have developed (1) methods to reconstitute native-like arrays of receptor cytoplasmic fragments, CheA, and CheW, and (2) a hydrogen exchange mass spectrometry (HDX-MS) method applicable to membrane-bound, multi-protein complexes. HDX-MS comparison of complexes with high and low kinase activity shows that differences localize to two functionally important subdomains of the receptor. Changes in the methylation subdomain that mediates adaptation are complex. The uniform exchange behavior of these peptides in the kinase-off complexes splits roughly in half in the kinase-on complexes, one fraction with slower exchange and the other with extremely rapid exchange (complete in 3 min). For the signaling subdomain that binds