limited by the size of the allosteric unit and not by the off-rate from the T-state. Thus, one driving force for the development of large allosteric units might be an increased plasticity in terms of modulation of ligand dissociation rates.
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2867-Plat
Multiscale Simulation of Intra-Protein Communication
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We develop a new computational framework to model intra-protein communication. The configurations sampled in atomic molecular dynamics trajectories are used to compute bond lengths and force constants in an elastic network approximation of the distribution of protein structures. To go beyond the harmonic approximation, a key novelty is to compute model parameters in consecutive time windows with a user-specified size to follow the time evolution of the mechanical coupling network of protein conformation. In analogy to spectrogram of sound waves, sequential elastic network models calculated from atomic trajectories are termed the fluctuogram of protein dynamics. By analyzing and comparing the fluctuograms of Ca2+-bound and apo subtilisin, we illustrate that intermittent conformational changes and mechanical coupling variation are important mechanisms of intra-protein communication. We also show that the fluctuogram can be used to predict residues with high tendency to co-evolve by comparing with the results of statistical coupling analysis of a multiple sequence alignment. In addition to the strength of mechanical coupling, we found that the fluctuation of inter-residue force constants is also an important descriptor for co-evolution. Together, the results of this work (a) reveal the intermittent nature of conformational changes and the mechanical coupling variation, (b) show that intra-protein communication can proceed without a drastic change of protein structure and the pathways of which can be identified by the fluctuogram, and (c) support the theory that mechanically coupled residues tend to co-evolve.

2868-Plat
Enhancing Allosteric Response in Thermus Thermophilus Phosphofructokinase
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Phosphofructokinase (PFK) from an extreme thermophile, Thermus thermophilus (TtPFK), exhibits 17-fold stronger binding to its inhibitor, PEP, and 34-fold weaker coupling between the binding of PEP and substrate, Fructose-6-phosphate (F6P), when compared at 250C to the PFK from another thermophile, Bacillus stearothermophilus (BsPFK). BsPFK is 57% identical in sequence. Since no 3-dimensional structural information is available for TtPFK, we turned to the crystal structures of BsPFK in search for the possible explanation. There is a network of residues, D59, T158, and H215, that leads from the allosteric site to the nearest active site, and that undergoes a significant rearrangement when PEP binds to free enzyme. In the apo form of BsPFK, H215 forms a hydrogen bond with T158. In the inhibitor-bound form, T158 is further removed from the allosteric binding site, and D59 forms a hydrogen bond with H215. In TtPFK these interactions are not possible due to nature of residues at these positions: N59, A158, and S215. We hypothesized that recreating this network of residues would strengthen the coupling between the PEP and F6P binding in TtPFK. Single amino acid substitutions at each of these positions resulted in some increase in binding free energy. The three combinations of double mutations produced a more significant increase in coupling free energy, which appeared in each case to be roughly the sum of the changes in coupling free energy produced by the individual mutants. Interestingly, the level of coupling attained by introducing all three mutations is similar to that seen in BsPFK, and the binding affinity of PEP was weakened to the level exhibited by BsPFK ($\Delta Gay = 3.98 \pm 0.03$ kcal/mol, $\Delta Gy = -5.61 \pm 0.01$ kcal/mol). Supported by NIH grant GM33216 and Welch Foundation grant A1548.

2869-Plat
Structural and Thermodynamic Origins of Ligand Specificity in Homologous PDZ Domains from the Tiam-Family of Guanine Exchange Factors
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PSD-95/DlgA/ZO-1 (PDZ) domains are among the most abundant protein-protein interaction domains in the human proteome and typically bind the 4-10 most C-terminal residues of its interaction partner with exquisite specificity. To investigate the origin of this specificity, we used two homologous PDZ domains from the Tiam-family of GEFs that have distinct but overlapping specificity for ligands. The Tiam1 PDZ domain binds 8-residue long C-terminal peptides derived from the proteins Syndecan1 and Caspr4 with micromolar affinity but does not bind Neurexin1. In contrast, the Tiam2 PDZ domain binds to peptides derived from Caspr4 and Neurexin1 with low micromolar affinity but not Syndecan1. Analysis of the X-ray crystal structure of the Tiam1 PDZ domain bound to a ''model'' peptide shows two specificity pockets created by four residues in the Tiam1 PDZ domain. Moreover, comparison of nuclear magnetic resonance (NMR) titrations of the Tiam1 PDZ domain with the Syndecan1 and Caspr4 peptides showed substantial differences in the changes in chemical shift in these residues. Sequence comparison of Tiam-family PDZ domains revealed that these residues are not conserved, further suggesting that they play a role in establishing ligand specificity. Double mutant cycle analysis of residues in these two pockets revealed ligand-dependent cooperativity, supporting their role in specificity is ligand specific. Remarkably, substitution of all four residues in the Tiam1 PDZ domain with the amino acids found in the Tiam2 PDZ domain switched the specificity to that of Tiam2. Collectively, our data suggest that Tiam-family proteins have highly evolved PDZ-ligand interfaces with distinct specificities, and that they have disparate PDZ-dependent biological functions.

2870-Plat
The Binding Energetics of a T-Cell Receptor Show a Bias Toward the Conserved Antigen Presentation Molecule, HLA
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T-cell receptors (TCRs) are heterodimeric receptors on the surface of T-cells with Complementarity Determining Region (CDR) loops similar to immunoglobulins. Their ligands are peptides presented by Major-Histocompatiblity Complexes (MHCs) on the surface of most nucleated cells. The TCR binds to an MHC presenting an antigenic peptide with a dramatically stronger affinity than MHCs presenting ''self'' peptides despite the fact that the majority of the interface is conserved between the two. To assess the energetic contributions of different portions of the TCR-pMHC interface, we quantify the contributions to binding of the side-chain contacts between the residues at the interface through alanine double-mutant cycles. The interaction energy between those residues is defined as native free energy change minus the free energy changes of the two single mutants, plus the free energy change of the double-mutant; ddGoint = dGo(Xwt-Ywt) - dGo(Xz->a-Ywt) - dGo(Xwt-Yz->a) + dGo(Xz->a-Yz->a). Our results for the A6 TCR and the tax9 peptide show that contrary to expectations, the contacts between the central CDR3 loops and the peptide do not have a unique energetic importance, but CDR3α has a ''hot-spot'' ~3 kcal/mol interaction with the MHC, HLA-A2. This interaction motif between a positively charged residue in the α1 helix of the MHC and a negatively charged residue in the CDR3α loop appears to be commonly utilized based on a comparison of TCR-pMHC x-ray crystal structures. Additionally, CDR1α and β both make significant hydrogen bonds to the peptide. These data show that the energetic basis for T-cell recognition is not parsed into recognition of the peptide by CDR3 and the MHC by CDR 1 and 2 but rather that TCRs bind a composite interface.

2871-Plat
Structural Basis of Ligand Recognition by the Tollip C2 and CUE Domains
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Toll-like receptors (TLRs) provide a mechanism of host defense responses by activating the innate and adaptative immune responses. Subsequent downstream events result in the recruitment of one or more adaptor proteins, a process mediated by the cytosolic tail of TLRs. These protein-protein interactions promote the activation of the interleukin-1 receptor-associated kinases (IRAKs) 1, 2, M, and 4 that act upon their transcription factor targets to influence the expression of genes involved in the innate immune response. The Toll-interacting protein (Tollip) controls IRAK function in the TLR signaling pathway. Tollip presents an N-terminal Tom1-binding domain, a central C2 domain, and a C-terminal coupling of ubiquitin to endoplasmic reticulum degradation (CUE) domain. We found that the Tollip C2 domain preferentially interacts with phosphoinositides including phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) in a calcium-independent manner. NMR and lipid-protein overlay analyses suggest that PI3P and $PI(4,5)P_2$ share the same binding site in the protein. Kinetic analysis reveals that the Tollip C2 domain reversibly binds PI3P and $PI(4,5)P_2$ with affinities in the low micromolar range. Mutational analysis identifies key phosphoinositide-binding basic residues in the Tollip C2 domain located in a flexible region nearby the beta-groove. The CUE domain binds ubiquitin, although the biological consequences of the association as well the molecular basis of the interaction are unknown. Using NMR spectroscopy, we have identified the Tollip CUE domain residues that recognize ubiquitin as well as the ubiquitin residues that bind to the Tollip CUE domain. Structural and kinetical analyses suggest that a dimeric Tollip CUE domain forms a complex with ubiquitin in conserved binding pockets with nanomolar affinity. Overall, our findings will provide the basis to understand how Tollip is intracellularly partitioned in a ligand-dependent manner and how these interactions modulate TLR signaling.