2001-Plat
Structural Studies of Ubiquitin and Ubiquitin-Like Protein Transfer Cascades
Brenda A. Schulman, David M. Duda, Hari B. Kamadurai, Judith Souphron, Daniel C. Scott, Julie K. Monda, Jennifer Olszewski, Matthew F. Calabrese.
St. Jude Children's Research Hospital / HHMI, Memphis, TN, USA.
Post-translational covalent attachment of ubiquitin-like proteins (Ubls) to protein targets is a predominant eukaryotic regulatory mechanism. In higher eukaryotes, more than a dozen Ubls - including ubiquitin, NEDD8, ISG15, and SUMO - covalently modify myriad substrates. The best understood function of a Ubl modification is ubiquitin-mediated proteasomal degradation. However, different Ubls alter the functions of their targets in different ways, such as by changing the target's subcellular localization, enzymatic activity, or interactions with other proteins or DNA. Our goals are to understand (1) the basic enzymatic mechanisms underlying Ubl attachment to targets, (2) how Ubls are attached selectively, and (3) how Ubl modifications alter target functions.
Ubls are attached to protein targets by the sequential action of enzymes in three classes, known as E1, E2, and E3. During this process, a Ubl becomes transiently covalently linked to enzymes, and ultimately to the target. This is a highly dynamic process, in which a Ubl is ''handed off'' first between enzymes, and ultimately to a target. I will present the latest research from my lab providing structural glimpses and/or biophysical analyses of molecular principles underlying dynamic protein regulation by ubiquitin and other Ubl transfer cascades.

2002-Plat
Characterizing Order and Disorder of Protein Structural Ensembles
Charles K. Fisher[1], Collin M. Stultz[2].
[1]Harvard University, Cambridge, MA, USA, [2]Massachusetts Institute of Technology, Cambridge, MA, USA.
The classical structure-function paradigm states that the amino acid sequence of a protein encodes a unique 3-dimensional structure that determines the protein's biological function. The view that most proteins adopt an ordered native conformation has been bolstered by the success of X-ray crystallography in determining high-resolution protein structures. Nevertheless, evidence from nuclear magnetic resonance spectroscopy, single molecule experiments and other techniques suggests that proteins exhibit a high degree of flexibility, which is critical for processes like molecular recognition. An information theoretic order parameter that describes the degree of heterogeneity in a protein conformational ensemble was used to characterize the flexibility of representative protein folds using computer simulations and a large sample of crystallographic B-factors from high-resolution protein structures in the Protein Data Bank. These data demonstrate that many proteins which are typically described by an ordered native structure, in fact, populate multiple conformational states.

2003-Plat
Structural Fluctuations and Conformational Changes in Proteins and Protein Complexes
Anatoly M. Ruvinsky[1], Tatsiana Kirus[1,2], Alexander V. Tuzikov[2], Ilya A. Vakser[1,3].
[1]Center for Bioinformatics, The University of Kansas, Lawrence, KS, USA, [2]United Institute of Informatics Problems, National Academy of Sciences, Minsk, Belarus, [3]Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, USA.
A systematic large-scale study of relationships between protein sequence, conformational changes upon binding, and residues fluctuations is important for better understanding of protein association. The results indicate that the scale of the residue fluctuations and conformational changes increases from the protein core to the surface. Residue mass, length and environment, determine the scale of the motions. On average, smaller residues undergo more significant fluctuations than the larger ones. The residues with one or two dihedral angles change conformations less than the residues with three or four dihedral angles. The relationship between the local conformational changes and the equilibrium fluctuations of a side chain around its unbound conformation is suggested.
Comparison of fluctuations and conformational changes of the binding site (interface) residues with other surface residues shows that the interface undergoes smaller fluctuations and larger conformational changes than the rest of the protein surface. The tendency of some residues to form more stable docking patches at the interface is discussed. Short and long residues typically follow different mechanisms of conformational changes. The long residues are more often subject to the induced fit resulting in a rotamer transition. The shorter residues generally undergo local conformational changes not leading to a rotamer transition.
Protein residues can be classified into highly, moderately, and weakly fluctuating, based on the normalized scale of fluctuations. The biased distribution of these groups in proteins supports a hypothesis that (a) structural instability in proteins relates to the high content of the highly fluctuating residues and the lack of the weakly fluctuating residues in protein loops, chameleon sequences and disordered proteins, and (b) the nucleation of the unfolded phase proceeds from protein loops.

2004-Plat
Orchestrating Population Shifts of Native Proteins in Different Environments
AliRana Atilgan, Deniz Turgut, Ayse Ozlem Aykut, Canan Atilgan.
Sabanci University, Istanbul, Turkey.
Conformational multiplicity of proteins under different environmental conditions is satisfactorily described herein by simple analytical methods. To efficiently monitor the equilibrium landscape and to orchestrate the population shifts, we derived the following set of equations originating from continuum mechanical considerations: i) the equilibrium equation of each repeating unit, ii) the constitutive relation for each (non)bonded short and/or long-range contact, and iii) the compatibility equation between the fluctuation of an element and fluctuations of its neighboring bonds. Together with the updated incremental Lagrange formalism, we put forward an effective single-molecule manipulation methodology. After each incremental move due to the perturbation induced by a ligand, the response kernel is updated and the new position on the free-energy surface is calculated.
In this study, we additionally demonstrate that cooperatively inserted intra-residue fluctuations, resembling different ligand insertions into the protein, moderate the positional motion of residues that are responsible for desired activities of the protein. We identify a feedback mechanism between sensory regions and adaptively distributed actuating parts. We construct a template that is a subset of the native structure containing the controller and we show that the template is conserved within the families of evolved sequences. These templates are shown to be the key elements for protein-protein interactions.
We study the relationships between the statistical and spectral properties of networks derived from the protein conformations located at the different minima of the landscape. We determine how the shortest path betweenness distribution of the edges is altered from one minimum (apo form) to another one (holo form). Furthermore, the results indicate that the changes in the redundancy index, which is the ratio of the number of alternative two-step paths a given residue i generates to its non-bonded contacts and its overall reachability, is significant.

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Perturbation Response Scanning Method for Identifying Allosteric Transitions and Utilizing in Flexible Docking
Z. Nevin Gerek, Ashini Bolia, S. Banu Ozkan.
Arizona State University, Tempe, AZ, USA.
We have recently developed coarse-grained method; perturbation response scanning (PRS) that couples elastic network models with linear response theory (LRT). It computes the response of the protein structure (i.e. displacement vector) upon exerting directed random forces on selected residues. The method has proven successful in reproducing residue displacements for a set of 25 proteins that display a variety of conformational motions upon ligand binding[1]. Using PRS we analyzed two PDZ domain proteins (PSD95 PDZ3 domain and hPTP1E PDZ2 domain) whose allosteric behavior play a key role in signaling. By PRS, we first identified the residues that give the highest response upon perturbing the binding sites. Strikingly, we observe that the residues that give the highest response agree with experimentally determined residues involved in allosteric pathways. Second, we constructed the allosteric pathways by clustering the residues giving same type of response upon perturbation of the binding sites. Interestingly our analysis provided molecular understanding of experimentally observed hidden allostery of PSD95. We have shown that removing the distal alpha helix from the binding site alters the allosteric pathway and decreases the binding affinity. Overall, these results indicate that (i) PRS is successful in capturing the conformational changes upon binding[1], (ii) it can identify key residues that mediate