943-Symp
Spatial Dynamics and Control of Cell Differentiation
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Understanding regulatory networks requires taking into account their controls and their spatiotemporal dynamics. We are probing these in experiments on the C. elegans germ line that target organ-level regulation of differentiation, and that leverage image segmentation workflows we have developed. In this talk I will explore the role of Notch signaling as a control of the germline regulatory network, and the role of diffusion in shaping the dynamics of the network. These are first steps towards our final goal of gaining a systems-level understanding of the C. elegans germline regulatory network.

PLATFORM Q: Protein Dynamics

944-Plat
Using Molecular Simulations to Understand Allosteric Inhibition of the Hepatitis C Virus RNA Polymerase
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The Hepatitis C Virus (HCV) affects more than 100 million people around the world. About a quarter of infected individuals will eventually contract chronic liver ailments and may suffer severe complications such as liver failure. There is no known cure for this disease and few effective treatments exist. The HCV RNA-dependent RNA polymerase (RdRp) is currently a target for small molecule therapeutics due to its importance in replicating the viral genome. Several allosteric inhibitors of RdRp have been identified which bind to the enzyme outside of the active site at which nucleotides are incorporated into newly synthesized RNA. While their mechanism is as yet unknown, these inhibitors have been suggested to act by preventing a conformational change in RdRp which is necessary to initiate RNA replication. We hypothesize that one can understand the nature of allosteric inhibition by using molecular simulations to study the dynamics of the enzyme, both in a free state and bound to different inhibitors. We seek to delineate the link between ligand binding and functionally important conformational fluctuations of RdRp by observing the structural coupling which results from the internal motions of the enzyme. In addition to answering fundamental questions regarding the mechanism by which allosteric effects can occur, these studies may provide information which can aid in the development of novel and more effective RdRp inhibitors.

945-Plat
Domain Fluctuations Enable Catalytic Activity in Phosphoglycerate Kinase?
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The biological function of enzymes is often related to large-scale domain movements. The configuration changes are observed by methods like x-ray crystallography, which give a static image of the protein structure in the crystal confinement. The question is, if these configuration changes are due to the substrate binding or if they are also related to the crystal packing which favors specific configurations. The structure of a protein in solution can deviate from the crystal structure but the protein has also the ability to fluctuate between different configurations. Are these fluctuations important for protein function?
Phosphoglycerate kinase (PGK) has a widely open domain structure with a hinge near to the active center between the two domains. The hypothesis of a substrate-induced configuration change, was first proposed by Banks et al. based on the comparison of crystal structures.
We have recently investigated the domain dynamics of PGK (1). Structural analysis by small angle neutron scattering revealed that the structure of the holoprotein in solution is more compact as compared to the crystal structure, but would not allow the functionally important phosphoryl transfer between the substrates, if the protein would be static. Brownian large scale domain fluctuations on a timescale of 50 ns was revealed by neutron spin echo spectroscopy. In particular, the domain movements facilitate a close encounter of the key residues in the active center to build the active configuration. The observed dynamics shows that the protein has the flexibility to allow fluctuations and displacements that seem to enable function. The presence of the substrates increases the rigidity, which is deduced from a faster dynamics with smaller amplitude.
(1) in press: Inoue et al., Large Domain Fluctuations on 50-ns Timescale Enable Catalytic Activity in Phosphoglycerate Kinase, Biophysical Journal (2010), doi:10.1016/j.bpj.2010.08.017

946-Plat
Coarse-Grained and Atomistic Modeling of Anisotropic Atomic Fluctuations in Protein Crystal Structures
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Protein atomic fluctuations can be probed by x-ray crystallography in the form of Anisotropic Displacement Parameters (ADP). In this study, we assess the accuracy of different coarse-grained and atomistic models that include protein-environment interactions in a protein crystal in comparison with experimental ADPs. We use a coarse-grained Elastic Network Model (ENM) with three different boundary conditions (see figure) to model protein-environment interactions, and an atomistic model using a CHARMM force-field. For a large list of high-resolution protein crystal structures, we find that optimal ADP modeling is achieved by weak protein-environment interactions as compared to internal interactions within a protein structure. Therefore, the internal dynamics of a protein is only weakly perturbed by crystal packing. We also find no improvement in the accuracy of ADP modeling by using the atomistic model over the coarse-grained ENM.

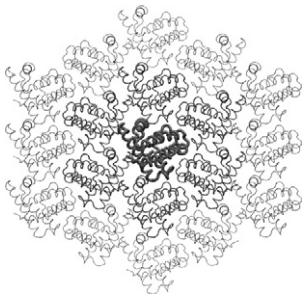

Figure. A protein-environment system constructed from a protein crystal of oxy-myoglobin (PDB code: 1a6m) with the main protein in red, the nearest neighbors in green, and the next nearest neighbors in blue. The main protein and the nearest neighbors are unconstrained while the next nearest neighbors are fixed.

947-Plat
Interconversion of Functional Motions Between Mesophilic and Thermophilic Adenylate Kinases
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Dynamic properties are functionally important in many proteins, including the enzyme adenylate kinase (AK), for which two small domains (LID and NMP) close over the larger CORE domain; the reverse (opening) motion limits the rate of catalytic turnover. Here, we compare our previously published coarse-grained (double-well G[[Unable to Display Character: ō]]) simulation of mesophilic AK from *E. coli* (AKmeso) to simulations of thermophilic AK from *Aquifex aeolicus* (AKthermo) in terms of the critical rigid-body, backbone dihedral, and contact motions in open, closed, and transition state (TS) ensembles. Like AKmeso, AKthermo follows a LID-first closure pathway in the presence of ligand, but the amplitude of LID rigid-body motions in the O ensemble decreases significantly. Backbone unfolding in O and/or TS ensembles decreases significantly relative to AKmeso in most of the interdomain hinges and within LID. In contact space, the TS of AKthermo has a weaker CORE-LID interface but a stronger contact network surrounding the CORE-NMP interface than the TS of AKmeso. A "heated" simulation of AKthermo at 375K and the simulation of AKmeso at 300K show similar conformational ensembles, both in the amplitude of CORE-LID motions in O ensemble and in the flexibility of some hinge regions, which supports the corresponding states hypothesis. Furthermore, mutation of 7 prolines unique to AKthermo to the corresponding residues from AKmeso more fully shifts the dynamics toward the more flexible behavior of AKmeso in most of the key hinges and even in some regions distant from any mutation. However, some prolines in AKthermo appear to strengthen or even substitute for nearby contacts from AKmeso so that local flexibility increases excessively upon mutating the proline. Finally, this mutagenic framework can inform the rational design of functionally important dynamics and allostery in other proteins toward engineering novel biological control systems.

948-Plat
Dynasome: How Does Protein Structure and Function Relate to Dynamics?
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Background: Proteins have been very successfully classified according to amino acid sequence or structure, which enabled improved prediction of function. In this study, we carried this idea one step further and developed a minimally biased scheme to compare and classify proteins purely according to their motion patterns. This approach is based on the notion that proteins, which fold into often recurring structural motives might also be exhibiting a distinct

set of recurring motion patterns. The complete set of these patterns, which we tentatively call the dynasome, spans a high-dimensional space whose axes, the dynasome descriptors, characterize different aspects of protein dynamics.
Methodology: The unique dynamic fingerprint of each protein is represented as a vector in the basis of this dynasome space. The difference between any two vectors, consequently, gives a reliable measure of dynamics similarity. From extended molecular dynamics simulations of 100 representatively chosen soluble proteins, dynamics fingerprints were obtained, which served to characterize in detail the statistical properties of the dynasome.
Conclusions: 1. We find that proteins do not fall into natural, well separated dynamics classes. 2. Four collective dynamics descriptors obtained from PCA are sufficient to characterize the dynasome. 3. For the majority of proteins we observe strong correlation between structure and dynamics. Exceptions are convergent and divergent dynamics, respectively, where minor structural differences yield major dynamics differences and vice versa. 4. Proteins with similar function carry out similar dynamics. Combination of structural and dynamics data yields superior predictions of protein function.

949-Plat
A Model Comparison for Characterizing Protein Motions from Structure
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To understand the extent structure plays in determining protein dynamics, a comparative study is made using three computational models that characterize native state dynamics starting from known protein structures taken from four distinct SCOP classifications. A geometrical simulation [1] (FRODA) is performed based on an initial rigid cluster decomposition using FIRST [2], and the results are compared to the commonly employed elastic network model (ANM) and molecular dynamics (MD) simulations. The essential dynamics is quantified by a direct analysis of a mode subspace constructed from ANM and a principal component analysis (PCA) on both the FRODA and MD trajectories using root mean square inner (RMSIP) product and principal angles (PA). Relative subspace sizes and overlaps are visualized using the projection of displacement vectors on the model modes. Additionally, a mode subspace is constructed from PCA on an exemplar set of X-ray crystal structures in order to determine similarly with respect to the generated ensembles. Our quantitative analysis reveals there is significant overlap across the three model subspaces and the model independent subspace. The subspaces generated from all three models were found to have high overlap for all four SCOP classes of proteins investigated, although FRODA provided the most robust sampling of the native basin. These results indicate that structure is the key determinant for native state dynamics. This work is supported by NIH grant 1R21HL093531.
[1] Wells S, Menor S, Hespenheide B, Thorpe MF: Constrained geometric simulation of diffusive motion in proteins. *Phys Biol*, **2**:S127-S136 (2005).
[2] Jacobs DJ, Rader A, Kuhn LA, Thorpe MF: Graph Theory Predictions of Protein Flexibility. *Proteins*, **44**:150-65 (2001).

950-Plat
Discovering Conformational Sub-States Relevant to Protein Function
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Native protein dynamics are governed by a hierarchical energy landscape, a multi-level configurational space whose hills and valleys correspond respectively to transition states and stable conformational sub-states. Internal motions enable proteins to explore this rugged landscape; increasingly, proteins are conceptualized as richly diverse ensembles rather than static structures. But the role of conformational fluctuations (or multiple conformations) in designated function of proteins is widely debated. Recent evidence indicates that sub-states of protein conformations exist containing both structural and dynamical features important for function. The low populations in these sub-states and the transient nature of conformational transitions have presented significant challenges for their identification and characterization. To overcome this challenge we have developed quasi-anharmonic analysis (QAA). QAA utilizes higher-order statistics of protein motions allowing identification of various states in the conformational hierarchy; further, the focus on anharmonicity allows the identification of conformational transitions between sub-states. QAA of equilibrium simulations of human ubiquitin and T4 lysozyme, elucidates a hierarchy of functionally relevant sub-states and protein motions involved in molecular recognition. In combination with a reaction pathway sampling method, QAA allows characterization of conformational sub-states associated with an enzyme reaction such as the cis/trans isomerization of peptidyl-prolyl amide bonds catalyzed by the enzyme cyclophilin A. In all three cases QAA reveals presence of a number of conformational sub-states at different levels in the hierarchy, with specific sub-states containing crucial structural and dynamical elements relevant for identification of binding other proteins (ubiquitin), binding substrate (lysozyme) and enzyme-substrate interactions in the active-site for the transition state formation and reaction mechanism (cyclophilin A). Overall, QAA provides a novel framework to intuitively understand biophysical basis of conformational diversity and its relevance to protein function.

951-Plat
Conformational Dynamics and Allostery of Supramolecular Protein Assemblies: from the Nuclear Pore Complex to GroEL
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Supramolecular protein assemblies participate in a broad range of cellular functions including transcription, translation, protein synthesis and folding, translocation of biomolecules, and cell division. Single particle cryo-electron microscopy is increasingly revealing the structure of diverse high molecular weight protein assemblies at ever higher resolution. While the static structure of these assemblies provides invaluable insight into their functional mechanism, important additional information is provided by their conformational dynamics. Here we present an unsupervised computational framework that is used to analyze the conformational dynamics of the majority of structures deposited in the Electron Microscopy Data Bank. Conformational dynamics are computed using normal mode analysis based on a recently established finite element framework, which is used to compute equilibrium thermal fluctuations, elastic strain energy distributions associated with specific conformational transitions, and dynamical correlations in distant molecular domains. Results are presented in detail for the nuclear pore complex from Dictyostelium discoideum and the chaperonin GroEL from Escherichia coli, revealing regions that are important to the stability of these molecules, as well as highly coupled dynamically in collective molecular motions. Results are publically available at http://www.cdyn.org and will be extended to include proteins in the Protein Data Bank, offering an important source of dynamical information that may be used to investigate the biological function of a broad range of molecules.

PLATFORM R: Membrane Structure II

952-Plat
Charachterizing Structure and Dynamics of Calcium-Induced Clusters of Phosphatidylserine in Mixed Lipid Bilayers
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The cellular membrane plays a key role in the regulation and activation of peripheral membrane proteins. For instance, the enzymatic activity of several coagulation factors and signaling proteins is regulated by their specific binding to negatively charged regions of the cellular membrane that are rich in anionic lipids such as phosphatidylserine (PS). The lipid composition of the membrane and the ionic content of the immediate solution significantly modify structural properties of the bilayer surface. In particular, calcium-induced clustering of PS lipids has been suggested to modulate membrane-protein interactions. We employ our novel highly mobile membrane mimetic (HMMM) model combined with molecular dynamics simulations to investigate structural and dynamic properties determining these interactions. The HMMM model, while preserving full representation of the lipid head groups that are required for detailed characterization of specific interactions, provides 1-2 orders of magnitude speed up in lipid mobility. Extended simulations with HMMM systems including anionic (POPS), zwitterionic phosphatydilcholine (POPC), or POPS/POPC binary mixtures provide a detailed view of structural changes that occur due to lipid-lipid and lipid-ion interactions, specifically those that drive PS clustering. Simulations revealed a diverse set of PC-PS-Ca2+ microdomains of consistent geometry. In particular, we observed 2PC:2PS:Ca2+:water and 2PS:Ca2+:3water stoichiometries. Ca2+ ions interact with phosphate groups of PC and PS as well as with the carboxy groups of PS. Interestingly, unimolecular chelation of Ca2+ by the same PS head group is often observed within the clusters. In contrast to monovalent Na+, the presence of divalent Ca2+ shows its long-lived coordination with lipid head groups that modulates their orientation and leads to formation of PS clusters. Prior to the development of the HMMM method these observations were out of reach of atomistic simulations.