Platform S: Protein Structure and Allosteric Communication

895-Plat AlloPathFinder: An Efficient Program Predicting Allosteric Communication via a Pathway of Conserved Residues

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We have developed a computer program that allows users to predict allosteric pathways in proteins. We explore the hypothesis that residues along the pathway of allosteric communication can be detected using only a combination of distance constraints between contiguous residues along the pathway and evolutionary data. To verify this hypothesis, we present a computational method that predicts a pathway of conserved residues connecting the myosin ATP binding site to the lever arm (Tang et al, 2007). We examined pre-stroke conformations of *Dictyostelium*, scallop, and chicken myosin II as well as *Dictyostelium* myosin I. In all these structures highly similar conserved pathways traversing switch II and the relay helix were observed, which is consistent with the understood need for allosteric communication in this conformation. We proceeded to apply our method to several post-rigor and rigor conformations across several myosin species and found very similar residues in these very different structures - especially along the relay helix leading to the beginning of the lever arm. Several mutational experiments had been performed to evaluate the significance of individual residues and we discuss and compare our results with these findings. Our method is remarkably simple, computationally efficient, and widely applicable. An easy to use application can be freely downloaded from https://simtk.org/home/allopathfinder and used to predict a set of residues that mediate allosteric communication or to simply find a path consisting of conserved residues between two locations in a protein structure.

References

Tang, S., Liao, J.-C., Dunn, A.R., Altman, R.B., Spudich, J.A., Schmidt, J.P. (2007). Predicting Allosteric Communication in Myosin via a Pathway of Conserved Residues, J. Mol. Biol., doi:10.1016/j.jmb.2007.08.059

896-Plat The Dynamics Energy Landscape Capturing Conformational Population Shift in Oligomerization

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Biochemical processes such as protein folding, binding, and catalysis often occur in microsecond to millisecond. Capturing dynamics motions of proteins in such slow timescale remains a challenging and pertinent goal for investigating the relation of structure, dynamics, and function. We present a multi-scale molecular dynamics approach that allows to sample large conformational changes and to identify important dynamic information in oligomerization. The method is tested on the β1 domain (the immunoglobulin-binding domain) of Streptococcal protein-G. Backbone dynamics order parameters computed using the model-free approach with a double exponential fitting function are highly correlated to those obtained from experimental residual dipole coupling measurements; this demonstrates the system is well sampled at the long term dynamics. Simulation results are analyzed and compared to motions that have been captured as possible mechanisms, explaining how monomer can be induced to form oligomers in agreement with protein engineering experimental studies. From the free energy calculations, conformational equilibria between monomeric and dimeric states as well as dimeric and tetrameric states are established. Conformational populations shift from tetrameric state to the dominant dimeric state, when the tetramer mutant (L5V/A26F/F30V/Y33F/A34F) is converted to dimerize by changing position of F26 back to the wild-type sequence A26.

897-Plat Networks of Local and Collective Motions Reveal Mechanochemical Coupling in a Wide Variety of Allosteric Proteins

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Allosteric proteins exhibit significant local and large-scale motions, but many questions remain about how such motions couple together to link substrate and effector sites. We previously found that network models based on tertiary structure changes (residue-residue contact rearrangements) alone account for substrate-effector connectivity in only a third of 15 allosteric proteins selected from a benchmark of 51 proteins. In this work, we develop the simplest possible representation for transitions between allosteric structures which captures both local and rigid-body motions and describes the allosteric coupling relationships arising from these motions. Hierarchical clustering of rigid segments based on a net rigid body motion distance metric partitions a protein structure into a set of rigid bodies. Graphs in which the nodes are rigid bodies, locally flexible regions, and substrate and effector ligands and the edges are atomic contacts between them define allosteric coupling relationships within the protein. Specifically, all nodes in a cycle are coupled, and any two cycles which share an edge are coupled. In 17 of 21 benchmark set proteins with spatially distinct substrate and effector sites, all substrate and effector sites are coupled into a single network, which is a substantial improvement over considering tertiary structure rearrangement alone. We also determine principal paths between substrate and effector sites, compare with crystallographers' analyses, and analyze the composition of rigid-body interfaces and the size distribution of rigid and flexible parts. In summary, these comprehensive allosteric transition models effectively account for substrate-effector communication and confirm the ubiquity of physical pathways between sites in allosteric proteins. These models may also be useful for biologically relevant coarse-graining of allosteric transitions in large proteins for simulation.

898-Plat Conformational Multiplicity and Landscape Shifts in Native Proteins

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We consider the folded protein as a network of its amino acids with links between residues in close proximity. The associated Hamiltonian is obtained from the residue network with the edges connected by equivalent springs. The criterion for proximity is based on a cut-off distance between central atoms on each residue. The spring constants are adjusted at different cut-offs by satisfying equipartition of the energy. The network structure is constructed unambiguously for each protein by selecting the cut-off distance minimizing the free energy (1). Using linear response theory, we reproduce residue-by-residue structural changes (2) as determined from the X-ray structures of the ligand-free and ligand-bound forms of example proteins (3). The method allows the analysis of very large proteins as well as domain motions. By sequentially inserting directed forces on single-residues along the chain (4) and recording the resulting relative changes in the atomic coordinates, we find that for the predominant number of the cases the residue-by-residue structural changes as determined from the X-ray structures are reproduced in detail (correlation coefficient larger than 0.9). Moreover, these changes are reversible, unless a ligand that introduces a few new interactions is also present in the model. The latter observations are explained by the incessant sampling of several conformational states - including that of the bound form - in the presence of fluctuating forces provided by the environment. Shifts in the energy landscapes are only induced once a ligand that stabilizes certain conformations is integrated to the system.

References

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899-Plat Long-Range Communication in Proteins identified Computationally

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We will present the results of computational modeling and analysis of two distinct protein systems in which long-range communication is functionally important. First, Mismatch repair proteins and second, peroxiredoxins.

900-Plat Oligomerization Of Membrane-bound Diphtheria Toxin (CRM197) Facilitates A Transition To The Open Form And Deep Insertion

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Diphtheria toxin (DT) contains separate domains for receptor-specific binding, translocation, and enzymatic activity. Following binding to cells, DT is taken up into endosome-like acidic compartments where the translocation (T) domain inserts into the endosomal membrane and releases the catalytic (C) domain into the cytosol. The process by which the enzyme is translocated across the endosomal membrane is known to involve pH-induced conformational changes, however the molecular mechanisms are not yet understood, in large part due to the challenge of probing the conformation of the membrane-bound protein. In this work neutron reflection provided detailed conformational information for membrane-bound DT (CRM197) in-situ. The data revealed that the bound toxin oligomerizes with increasing DT concentration, and that the oligomeric form (and only the oligomeric form) undergoes a large extension into solution with decreasing pH that coincides with deep insertion of residues into the membrane. We interpret the large extension as a transition to the open form. These results thus indicate that as a function of bulk DT concentration, adsorbed DT passes from an inactive state with a monomeric dimension normal to the plane of the membrane to an active state with a dimeric dimension normal to the plane of the membrane.

901-Plat Geometry-based Sampling of Conformational Transitions in Proteins

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The fast and accurate prediction of protein flexibility is one of the major challenges in protein science. Enzyme activity, signal transduction and ligand binding are dynamic processes involving essential conformational changes ranging from small side-chain fluctuations to reorientations of entire domains. In the present work we describe a re-implementation of the CONCOORD approach, termed tCONCOORD, which allows a computationally efficient sampling of conformational transitions of a protein based on geometrical considerations. Moreover it allows to extract the essential degrees of freedom, which in general are the biologically relevant ones. The method rests on a reliable estimate of the stability of interactions observed in a starting structure, in particular those interactions that change during a conformational transition. Applications to adenylate kinase, calmodulin, aldose reductase, T4-lysozyme, staphylococcal nuclease and ubiquitin show that experimentally known conformational transitions are faithfully predicted.

902-Plat Computational Modeling of Conformational Change in Allosteric Proteins

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It has been more than 40 years since Koshland et al., and Monod et al., put forth their models to explain the observed behavior of

allosteric proteins. While both models fit biochemical data and serve as useful conceptual tools, neither describes the atomic details of the mechanisms responsible for how allosteric proteins switch from one conformational state to another. Motivated by recent advances in high-resolution modeling, we set out to develop a method that predicts a protein's alternative state from a starting crystal structure. This method allowed us to probe the atomic details underlying conformational switching. To test this method, we chose proteins whose conformational change, both large and small, were induced by an external trigger such as ligand binding. We hypothesized that removal of the trigger would destabilize the starting structure, making the alternative, "unbound" state lower in energy, and thus more favorable. Given sufficient conformational sampling, we proposed that lower-energy models would represent the alternative structure. Here we demonstrate a method for predicting alternative structures of allosteric and non-allosteric proteins with an accuracy of 1 Ã... root mean square deviation (RMSD) to the experimentally determined structure. In the process of predicting these alternative states, we generated a large set of conformations that map a potential energy landscape. Conformations within this landscape clustered into near-native structures, suggesting these proteins behave like a two-state system. By dividing the energy landscape into the near-native (starting and alternative) and far-from-native structures, we were able to identify residue pairs that predict the structural transitions in switching between conformational states. The combination of predicting unknown alternative conformational states and identifying important contact pairs that drive these conformational changes presents an important advance in computational modeling of allosteric proteins.

Platform T: Muscle Mechanics & Ultrastructure

903-Plat Force depression in single myofibrils

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Introduction

It is well accepted that the steady-state isometric force following shortening of an activated muscle is smaller than the corresponding steady-state force obtained for a purely isometric contraction at the corresponding length. This phenomenon is referred to as force depression. The mechanism underlying force depression is not well understood. However, it has been suggested that a decrease in the number of attached cross-bridges might be responsible for the loss of force after shortening. In this study, we wanted to gain further insight into force depression by testing whether force depression occurs in single myofibrils and whether force depression is associated with a decrease in myofibril's stiffness.

Materials and Methods

Myofibrils (n=11) were activated at an average sarcomere length of 2.8μm and then shortened to an average sarcomere length of 2.4μm. In order to measure the stiffness of activated myofibrils after shortening, a quick stretch-release cycle was imposed to myofibrils before deactivation. Myofibrils were then reactivated at the sarcomere length of 2.4μm in order to obtain the isometric reference force and associated stiffness.

Results and discussion

Shortening of myofibrils produced force depression in all eleven myofibrils of (mean±SEM) 30.9±3.9% of the reference force. Furthermore, there was a decrease in stiffness after shortening of 30.2%. We conclude from these results that force depression is a sarcomeric property and that it is caused exclusively by a decrease in the proportion of attached cross-bridges, rather than a decrease in the force per cross-bridge. The mechanisms responsible for the shortening-induced decrease in the proportion of attached cross-bridges remain unknown. At present, we speculate that shortening causes an increase in the rate of cross-bridge attachment, thereby reducing the duty ratio in a shortening-magnitude and force dependent way.

904-Plat Critical Sarcomere Length Extension And Phase Transition Of Force During Lengthening Of Skeletal Muscle Myofibrils

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When skeletal muscle is stretched while activated, force increases in two phases. The purposes of this study were to evaluate the critical stretch for attaining the force transition in individual sarcomeres, and to test the hypothesis that stretch forces are produced largely by pre-powerstroke cross-bridges. Rabbit psoas myofibrils were attached between a pair of microfabricated cantilevers and a glass needle connected to a motor arm, activated, and stretched (~5% sarcomere length) at velocities ranging between 0.25 to 5.5 $\mu m \cdot sec^{-1}$ (~0.10 to 2.4 $L_o \bullet sec^{-1}$). Sarcomere length showed significantly dispersion at rest and activation (maximal dispersions of 35.4 nm and 76.0 nm, respectively), which increased at the transition point (84.2 nm) and reached the maximum at the end and just following stretch (131.9 nm and 159.6 nm, respectively). When stretch was performed at 0.5 $\mu m \cdot sec^{-1}$, the transition between the two phases occurred at an average critical stretch (SLc) of 7.0 ± 0.6 nm/half sarcomere, but the critical stretch varied considerably among sarcomeres (from 2 to 14 nm/half sarcomere). The force attained at the critical stretch (Fc) was 1.69 ± 0.24 times the isometric force produced before stretch. Stretch velocity did not affect SLc, but the F_c increased with increasing velocities up to 2.0 μm/s. 2,3-Butanedione monoxime (BDM), which biases cross-bridges into pre-powerstroke states, did not significantly change the SLc (8.5 ± 0.3 nm/half sarcomere). BDM decreased the isometric force to 21.45 ± 9.22% of the isometric force, but it increased the relative F_c to 2.21 ± 0.34 times the isometric force (at 0.5 $\mu m \bullet sec^{-1}$), suggesting that pre-powerstroke cross-bridges contribute to the stretch forces.

905-Plat Atomic Force Microscopy Reveals Details of Myofibril Architecture

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