View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

are believed to be contributed by the rotational motion of the side methyl groups [3]. Distinguishable dynamical behavior found between two proteins reveals local flexibility and conformational substates unique to oligomeric structures. Our results greatly help understanding the relation between protein dynamics and their biological functions.

[1] X.-Q. Chu, et al, JPCB. 116, 9917 (2012).
[2] X.-Q. Chu, et al, Soft Matter 6, 2623 (2010).
[3] X.-Q. Chu, et al, JPCL. 4, 936 (2013).

3289-Pos Board B17

Allosteric Regulation of Protein Kinase Enzymes via an Electrostatic Switch that Modulates Active Site Dynamics

Matthew A. Young[1], Douglas M. Jacobsen[2], Zhao-Qin Bao[1].
[1]Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA, [2]Bioinformatics, University of Michigan, Ann Arbor, MI, USA.

Protein kinase (PK) enzymes are a large family of signaling proteins that play a central role in signal transduction pathways. Robust regulation of their catalytic activity is critical, and many oncogenes harbor mutations that result in misregulated PK activity. The chemical basis for how some PK regulatory factors ultimately affect the rate of chemistry is still not completely understood. We have identified a long-range electrostatic switch that we believe is used by allosteric PK regulatory factors to modulate the rate of chemistry by tuning active-site dynamics.

We applied a combination of crystallography, kinetics, and molecular dynamics to determine the chemical kinetic basis for how this electrostatic switch, toggled by regulatory subunit binding, affects each step of catalysis by CDK2 kinase. We engineered point mutants to deconstruct the kinetic, dynamic, and thermodynamic consequences of the switch. We also evaluated other PKs and find that, although it has evolved to be triggered in different ways by diverse PK regulatory factors, the mechanics of this switch can be conserved.

We demonstrate that a key component of the switch is that it affects a significant change in the electrostatic potential within the ATP*Mg binding site of the enzyme. This electrostatic effect is propagated through the low-dielectric protein interior and directly affects the two dominant rate-determining steps of catalysis: attenuating both the recruitment of catalytically essential Mg cofactors (affecting both kcat and KM) as well as the release of the ADP product.

Conclusion: We present a chemical hypothesis that provides a mechanistic explanation for one way that a large-scale conformation transition, observed in diverse PK family members, is able to significantly affect the rate of chemistry by acting at a distance from the active site.

3290-Pos Board B18

A Network of "Molecular-Switches" Control the Activation of Key Bacterial Signaling Protein

Dan Vanatta.
Stanford University, Stanford, CA, USA.

Recent successes in simulating protein structure and folding dynamics have demonstrated the power of molecular dynamics to predict the long timescale behavior of proteins. Here, we extend and improve these methods to predict conformational change pathways between active and inactive state of nitrogen regulatory protein C (NtrC). By employing unbiased Markov State Model based molecular dynamics simulations, we predict a new dynamic picture of the activation of a key bacterial signaling protein NtrC, involving a complex network of molecular switches. These results are consistent with experimental observations and predict new mutants that could be used for validation of the mechanism. Moreover, these results suggest a novel mechanistic paradigm for conformational switching.

3291-Pos Board B19

Coarse-Grained Modeling of the Dynamics and Allosteric Modulation of Hras Protein

Abhijeet Kapoor, Alex Travesset.
Iowa State University, AMES, IA, USA.

HRAS acts as molecular switch by cycling between active (GTP-bound) and inactive (GDP-bound) state during signal transduction processes associated with cell growth and differentiation. The wealth of biochemical and structural data available for HRAS has identified critical regions in protein structure that plays crucial role in signalling. However, the mechanism by which active and inactive state transition occurs is not yet completely understood due to lack of experimentally determined intermediate structures. Also, the timescale at which these processes occur is currently beyond the reach of all-atom molecular dynamics simulations. In this talk, I will describe the dynamics of GDP and GTP bound form of HRAS with and without GEFs, using a transferable intermediate resolution model developed by us. In the model, the backbone is represented with atomic resolution but the sidechain with single bead and it has sufficient predictive power so that– Starting from random initial configurations, the model properly folded 19 proteins (including a mutant sequence) in to native states containing β-sheet, α-helices and mixed α/β. The model is then used to predict the dynamics of HRAS. The predictions of the coarse-grained model are tested with different 100 ns simulations. We present intermediate states and demonstrate, among other results, that the opening of Switch I/β-2 region in HRAS-GTP is a thermally activated process and occurs in the absence of GEF.

3292-Pos Board B20

Redistribution of Flexibility in Stabilizing Antibody Fragment Mutants Follows Le Chatelier's Principle

Tong Li[1], Malgorzata B. Tracka[2], Shahid Uddin[3], Jose Casas-Finet[4], **Donald J. Jacobs**[5], Dennis R. Livesay[1].
[1]Bioinformatics and Genomics, UNC Charlotte, Charlotte, NC, USA, [2]Formulation Sciences, MedImmune Ltd., Cambridge CB21 6GH, United Kingdom, [3]Formulation Sciences, Department of Formulation Sciences, Cambridge CB21 6GH, United Kingdom, [4]Analytical Biochemistry, MedImmune LLC, Gaithersburg, MD, USA, [5]Physics and Optical Science, UNC Charlotte, Charlotte, NC, USA.

Proteins exhibit rich dynamic behavior, yet protein structure is highly constrained by cross-linking H-bonds. This dichotomy makes it difficult to predict the effect of individual mutations on protein thermodynamics and dynamics. For example, it is often perceived that stabilizing mutations cause a protein to become more rigid due to improved packing. However, due to the complex relationships between rigidity and thermodynamics, mutations can cause unexpected long-range effects such as entropic stabilization in conjunction with increased dynamics. In general, mutations shift the rigidity-flexibility equilibrium within the native state ensemble. We quantify these shifts across a handful of stabilizing mutants within the single chain F_v (scFv) anti-lymphotoxin-β receptor antibody fragment using all atom explicit solvent molecular dynamics simulation, where the MD trajectories over 100 ns are analyzed by a Distance Constraint Model. Despite all of the mutants being stabilizing, we observe a statistically significant redistribution of rigidity/flexibility that extends far from the mutation. For the scFv fragments, we find that an increase in flexibility is much more common than an increase in rigidity. Interestingly, a net gain or loss in flexibility of an individual mutant is typically skewed. The redistribution of flexibility can be mechanistically traced to changes in the H-bond network. For example, the formation of new H-bonds due to a stabilizing mutation will generally induce a local increase in rigidity, while at the same time H-bonds break elsewhere, causing a frequent increase in flexibility far removed from the mutation site. Increased flexibility within the VH β-four and β-five loops is a noteworthy illustration of this long-range effect. As a general rule of thumb, we suggest that rigidity-flexibility equilibrium shifts manifest themselves through enthalpy/entropy compensation in the native state ensemble as the protein structure adjusts via Le Châtelier's principle.

3293-Pos Board B21

Generalized Model-Free Spectral Density Analysis Applied to Rhodopsin Activation in Membranes

Xiaolin Xu[1], Andrey V. Struts[2,3], K.J. Mallikarjunaiah[2], Michael F. Brown[1,2].
[1]Department of Physics, University of Arizona, Tucson, AZ, USA, [2]Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, [3]Division of Medical Physics, St. Petersburg State Medical University, St. Petersburg, Russian Federation.

Although molecular structures of G-protein-coupled receptors (GPCRs) are becoming increasing available from X-ray crystallography, understanding their functions requires information about molecular dynamics in membranes. Here we use rhodopsin as a model to illuminate general features of GPCR activation. With solid-state ^{2}H NMR spectroscopy we obtain experimental data pertinent to both structure and dynamics. Experimentally, order parameters and relaxation rates are the two observables of solid-state ^{2}H NMR experiments. We propose that the local dynamics of the retinylidene ligand are coupled to large-scale fluctuations of the transmembrane helices of rhodopsin, leading to activation of the receptor. To study the structural dynamics of retinal bound to rhodopsin, we start with an irreducible representation of the correlation function in terms of mean-squared amplitudes and correlation times [1]. The mean-squared amplitudes are related to the orientational order parameter, while the irreducible correlation times include the preexponential factor and energy barrier. To bridge the generalized model-free theory with experimental measurements, we separated the relaxation rates into spectral densities by applying Redfield theory. The spectral