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Protein Conformation I

346-Pos Board B115

Molecular Dynamics Study of Phosphorylation Mediated Structural Changes in Neurofilament Medium (NF-M) Subunit

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Neurofilaments (NFs) are essential building blocks of axonal architecture. Abnormal behavior of these cytostructural elements has been associated with several neuromuscular disorders such as Amyotropic Lateral Sclerosis (ALS). NFs are assembled from three subunits: Low (NFL), Medium (NFM) and Heavy (NFH). These subunits are characterized by a common alpha helical rod domain and carboxyl terminal domains of different lengths specific to each subunit. The tails project from the core of the filament and contain a number of KSP repeat motifs that belongs to the sites for phosphorylation. Especially, the C-terminal tails of NFM and NFH that have relatively longer lengths and higher number of KSP repeats were found to be the key participants of the sidearm-mediated interfilament interactions that regulate the axonal diameter. Though it has been established that that the sidearms play a key functional role, little is known about the roles of individual subunits and the effect of phosphorylation on their behavior. Initially, it was believed that the NFH sidearms play a more integral role in determining axonal structure due to the presence of longer polypeptides and relatively higher KSP repeat units. However, recent studies showed that deleting NFH from neurofilaments does not affect axonal diameter, suggesting that NFM may in fact be the key player. In view of this, it is essential to have an understanding of the morphological behavior of the NFM sidearm in response to physiological conditions. In the present study we carried out MD simulations of human and mouse NFM C terminals under different phosphorylation and ionic conditions. The results from these studies provide useful molecular level insight into the structural changes of NFM sidearms in response to phosphorylation, ionic concentrations. The present study reveals sidearm-mediated regulation mechanism of axonal caliber.

347-Pos Board B116

Structural Determinants of Conformational Flexibility and Long-Range Allostery of the CRM1 Export Complex

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In eukaryotes the nucleocytoplasmic transport of macromolecules is mainly mediated by soluble nuclear transport receptors of the karyopherin-β superfamily termed importins and exportins. The prototypical and highly versatile exportin CRM1 (chromosome region maintenance 1) is essential for nuclear depletion of numerous structurally and functionally unrelated protein and RNP cargoes. CRM1 has been shown to bind RanGTP (GTP bound RAs-related nuclear protein) and cargo proteins in an allosteric manner and to adopt a toroidal structure in several functional transport complexes. It was thought to maintain this conformation, with N- and C-terminal regions in close proximity, throughout the entire nucleocytoplasmic transport cycle.
A recently solved structure of cargo-free CRM1 however revealed a superhelical, open conformation. Using molecular dynamics simulations, two distinct features of this conformation and their influence on the structural stability were investigated. One of those, the C-terminal helix, was identified as a major stabilising factor of the superhelical conformation.
We furthermore showed that the overall configuration of CRM1 influences the local configuration of the cargo binding site. Based on these results we suggest a mechanism for the observed cooperative binding.

348-Pos Board B117

Characterizing the Molecular Mechanism of the Histidine Switch Model in Influenza Virus Hemagglutinin

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Hemagglutinin is a specific homotrimer glycoprotein on the surface of influenza virus envelope that consists of two subunits, HA1 and HA2. pH-mediated conformational changes of the HA2 chain play a key role in membrane fusion of the viral envelope to the host cell endosomal membrane. Two major steps are involved: first, formation a needle-shaped structure that inserts into the endosomal membrane from the N-terminus; second, re-bending of HA2 at a hinge region (residues 106 to 111) into a hairpin-shaped structure that brings the viral envelope very close to the endosomal membrane, thereby fusion of the two membranes. Following the histidine switch hypothesis, in order to characterize the molecular events taking place in the hinge region of HA2 in response to pH changes, we have performed molecular dynamics (MD) simulation of several hemagglutinin subtypes at neutral and low pH conditions, modeled by changing the protonation state of a histidine side chain located in this region. More than sixty sets of MD simulations (collectively amounting to 20 μs) of a 26-residue representation of the hinge-region were performed in implicit and explicit solvents to study the effects of histidine protonation. Bending of the hinge was observed upon protonation of the histidine in all models with an initial straight conformation, whereas the models with neutral histidine retained their primarily straight conformation. The MD simulations starting from an initially bent conformation resulted in the formation of a straight helical structure upon neutralization of the histidine, while the bent structure was maintained in the presence of a protonated histidine. Finally, mutation of the key histidine to alanine completely abolishes the bending of the peptide altogether.
Our results showed that histidine protonation is critical for low-pH conformational changes of the hinge region in HA2.

349-Pos Board B118

Recognition of Chemotherapeutically Damaged DNA by Mismatch Repair Proteins

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Complementing scarce experimental data, we provide computational evidence via all-atom molecular dynamics simulations for conformational changes and dynamical changes induced in the MSH2/6 heterodimer in response to DNA damage induced by platinum-based chemotherapuetics. We demonstrate thay 1,2 and 1,3 intra-strand platinum-DNA adducts are recognized by MutSα in a similar manner, but with subtle differences which may play a role in the way the different damages are signaled by MutSα.

350-Pos Board B119

Benchmarking the Water-Peptide Interaction

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The interaction between water molecules and the hydration sites of peptides is critical for any quantitative modeling of solvated peptides. We address this interaction for the successive hydration of two peptides for which accurate experimental reference data exist:Ac-Ala_5-$LysH^+$ (non-helical) and Ac-Ala_8-$LysH^+$ (helical). In particular, finite-temperature Gibbs reference water binding energies ΔG^0 and equilibrium constants are known [1,2]. In contrast, earlier force-field predicted preferred water binding sites do not agree with one another. We present an exhaustive first-principles study (density-functional theory based on the van der Waals corrected PBE functional) that demonstrates: (i) There is a close competition between possible hydration sites (protonated carboxyl group or ammonium group). The preferred first hydration site breaks an intramolecular bond of the ammonium group in the unsolvated molecule. (ii) Calculated $\Delta G^0(T)$ are in remarkable agreement with experimental data. Lowest-energy H_2O H-bond networks are predicted for up to five H_2O molecules, and the connection to the solvated state is explored by ab initio molecular dynamics with up to 152 H_2O molecules.
[1] Int. J. Mass Spec. 236, 81 (2004)
[2] J. Am. Chem. Soc. 126, 8454 (2004).

351-Pos Board B120

Predictive Power of Conformational Motion

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Biological macromolecules are flexible and dynamic systems, continuously changing shapes in response to environmental or other factors. Each possible shape is called a conformation, and a transition between them is referred as a conformational motion. The conformational motion may be induced by many factors which in a sense of theoretical physics should be explained by equation of conformational motion. In previous presentation (Svintradze D.V. (2009) Conformational Motion of Biological Macromolecules. Biophys. J. 96, 584) we pinpointed the possibility of formulation the equation of conformational motion which would go beyond of biophysics and touch fundamental problems of physics. In upcoming presentation we would like to apply the equation to proteins and DNA and deduce fluctuation frequency of the macromolecules. Fundamental Theory has to be uniformly true for all dimensions and should extend current knowledge of physics so that equivalence principal has to hold good. The theory of conformational motion has such potential. In order to rigorously clarify the point we will derive Einstein field equation from equation

of conformational motion and discuss some simplified cases. In principal will be shown that in a case of first approximation when the field equation holds good with cosmological constant than macromolecular surfaces undergo to vibrational motion and the frequency of such oscillations directly depends on Ricci scalar. In another words when linear configuration of Einstein tensor and metric tensor is proportional to energy stress tensor then equation of conformational motion reduces to simplified equation similar to Hooke's low rewritten in tensorial form and has well defined mathematical solution. Correspondingly the question why biological macromolecules do not have single energetic minimums and fluctuate among many energetic minimums will be answered.

352-Pos Board B121

pH-Dependent Free Energy Landscape, Conformational Selection, and Thermodynamics of Protein Folding

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Protein conformation change depending not only on the values of temperature, denaturant concentration but also on the values of solvent pH. The difference of the pH-denaturation from the thermal or urea denaturation is that hydrogen atoms (un)bind exclusively to R, K, Y, C, H, D, E amino acids. Thus the pH effect on the protein conformation is selective so that the physico-chemical machinery for the biological function of a protein frequently has its origin due to the solvent pH. Although several previous approaches were suggested to elucidate the (un)protonation behavior of a protein conformation, those were mainly oriented on evaluating pKa values of titratable residues in a given static protein conformation. The theoretical and calculation framework for describing the effect of solvent pH to the thermodynamic and kinetic properties of proteins under the equilibrium fluctuation is indispensable for the fundamental understanding of important biological phenomena of proteins.

Here we present a development of the pH-dependent free energy function of proteins incorporating its equilibrium fluctuations based on the concept of statistical physics. The validity of our approach is justified by reproducing the experimental pKa values of titratable residues in several proteins. We also present the analytical and calculation framework for describing the pH-dependent thermodynamics and folding kinetics of proteins by the exact calculation. The effects of pH not only on the free energy landscape but also on the folding characters of several proteins are discussed.

353-Pos Board B122

Functional Properties of HIV1 Reverse Transcriptase from Normal Mode Analysis with Elastic Networks and Essential Dynamics

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All DNA Polymerases possess similar spatial features, functional properties, and have similar discrete state kinetic mechanisms to describe function. One of the fundamental goals in biophysics is to predict the mechanism, and eventually, the dynamics of protein function, from purely structural information. To this end, normal mode analysis is a well-established first step. In fact, the lowest frequency normal modes of proteins often correspond to the largest amplitude conformational changes, and are thus likely to play a dominant role in a protein's functional properties. We have both qualitatively and quantitatively explored the low frequency modes for the enzyme HIV1 Reverse Transcriptase, with and without DNA bound to the polymerase active site, using an Elastic Network model. We then compared the Elastic Network modes to those calculated from Essential Dynamics of nanosecond scale all-atom molecular dynamics simulations. From these comparisons we have isolated specific large amplitude modes of the protein corresponding to the fingers closing, in addition to other torsional oscillations, and assess equilibrium states for both free polymerases as well as those bound with dsDNA.

354-Pos Board B123

Exploring Macromolecular Machine Motions

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Normal modes in internal coordinates (IC) furnish an excellent way to model functional collective motions. Here we present an enhanced version of our versatile NMA-IC framework, iMod (1). Even though the complexity reduction obtained from the IC and the employ of coarse-grained (CG) representations, the diagonalization step remained as a bottleneck for large macromolecular machines. Now, virus, long F-actin filaments or large microtubules can be studied with moderate CG representations by solving the large-scale eigenvalue problem on shared-memory multiprocessors using ad doc algebra procedures. Also, new parameterization of the elastic model has been done to improve the overall conformational flexibility description. By extending its applicability to larger systems and by improving elastic network potentials, we expedite the study of the collective conformational changes of such biological relevant complexes and their functional implications.

1.López-Blanco JR, Garzón JI, Chacón P. (2011) iMod: multipurpose normal mode analysis in internal coordinates. Bioinformatics. 27 (20): 2843-2850.

355-Pos Board B124

Conformational Dynamics of Ras Isoforms: Specificity at the Catalytic Domain

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Ras proteins serve as crucial signaling modulators in cell proliferation through their ability to hydrolyze GTP and cycle between GTP ''On'' and GTP ''off'' states. There are four different human Ras homologues and the sequence homology is almost conserved at the catalytic domain. These homologues differ in their ability to activate different effectors and hence different signaling pathways. Much of the previous work on Isoform specificity has attributed this difference to the HVR region of Ras proteins which dictates its localization in the membrane. In this work, we have analyzed the specific dynamics in the catalytic domain of two Ras Homologues H-ras and K-ras to probe for alterations in the active site architecture that could possibly provide effector and modulator specificity to the different isoforms. We explored the conformational dynamics between of WT H-ras and K-Ras proteins and compared the conserved communications and residue interactions between these two proteins at the catalytic domain. We have also studied the dynamics of a transforming mutant of H-ras and K-ras and an effector selective??? mutant of H-ras. Preliminarily analysis revealed that there is a distinct conformational distribution for K-ras and H-ras, including in the functionally important switch regions. Collectively we have determined that wild type K-ras is more dynamic than H-ras and that the structure of the effector binding loop more closely resembles that of the T35S Raf-selective mutant, providing new insight into the mode of effector specificity at the catalytic domain. Furthermore we have determined that specific mutations in H-ras and K-ras perturb the conformational equilibrium differently.

356-Pos Board B125

Detailed Conformational Changes Involved in TopoII DNA Binding, Bending and Cleavage

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We have used Molecular Dynamics (MD) simulation methods and two analytical approaches(the Gaussian Net- work Model(GNM) and Anisotropic Network Model (ANM)) to investigate the structural and energetic details of the S.cerevisiae topoII during the first step of its catalytic cycle. At the initial state of the first step of its catalytic cycle, the protein and 34 bp straight-DNA structure have no interaction. At the final state of the cycle, we have the bended-DNA/TopoII complex where the protein binds to DNA. The results show that DNA-gate and C-gate opening/closing mechanism causes the DNA-bending before the DNA G- gate cleavage. There is strong agreement between the theoratical and the experimental DNA-bending results where its global bending is $\approx 150^{\wedge}0$.The results also show that there is a hysteresis between DNA-bending and gate openings/closings. The transition of 3 helices on Winked Helix Domain during DNA bending and cleavage states has been also investigated because this transition might be important for the T-segment DNA passage through the G-Segment DNA. Normal mode analysis is additionally used to characterize the functional flexibity of the protein,especially C-gate domain closing/opening during the DNA binding process and before its cleavage.The Plastic Network Model(PNM) is also used to generate a conformational change pathway for S.cerevisiae topoII based on two (C-gate closed and open) crystal structures. PNM connects the energy basins corresponding to known two crystal structures at their lowest common energies. Analysis of PNM provides an identification of hinge motion upon DNA binding/bending. We also showed that 'trapdoor' mechanism causes faster closing of C-gate domain of the protein than the closing of the same domain without TYR residue upward motion. Because of its clinical importance, our study may provide new insight into the dynamics and structure of TopoII-DNA complex.

357-Pos Board B126

Conformation-Switching in Adenylate Kinase Revisited with a Path-Ensemble Simulation

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Adenylate kinase (ADK), which reversibly converts ATP and AMP to two ADP molecules, has two conformational states, inactive (open) and active (closed); crystal structures of both states were solved in mid 1990s. Numerous studies using experiments as well as computer simulations have aimed to elucidate the relationship between conformational rearrangement and ADK function.