Posters

Protein Structure and Conformation II

933-Pos Board B1

Conformational Dynamics of Histone Lysine Methyltransferases by Millisecond-Timescale Molecular Dynamics on Folding@home

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Epigenetic regulation is essential for eukaryotic organisms in processes spanning from embryo development to longevity. Histone lysine methyltransferases (HKMTs) are amongst the key players that control these processes. HKMT dysregulation via mutation or altered expression has been implicated in many cancers' initiation, maintenance, aggressiveness and metastasis. Furthermore, roles of HKMTs in aging and drug addition have been shown in animal models. Development of selective inhibitors for many members of this protein family remains an unmet need. Conformational dynamics have been observed or proposed at both cofactor- and substrate-binding sites of most HKMTs; this structural plasticity has a crucial impact on the shapes and druggabilities of pockets in HKMTs and on inhibitor design. Here we will present multiple-millisecond aggregate timescale Molecular Dynamics simulations, collected on Folding@home, for the SETD8, SETD2, NSD1, NSD2 and NSD3 methyltransferases. All these proteins were simulated in the *apo* form, and Markov State Models were constructed to map the thermodynamic and kinetic landscapes of the conformational ensembles. Furthermore, hypotheses for the dynamics within the catalytic cycle of the SETD8 methyltransferase, based on the available and two new crystal structures, were tested by Molecular Dynamics. In addition to the *apo* simulations, 'chimeric' homology models (assembled from domains of the protein from multiple crystal structures) were constructed and propagated in simulations; moreover a whole-catalytic-cycle set of simulations, comprising all possible combinations of the co-factor SAM, by-product SAH and histone H4 peptide, were conducted. Here we present a complete model of the catalytic cycle of the SETD8 methyltransferase, based on ~5 ms aggregate simulation time. Furthermore, verification of the computational results via biochemical experiments is presented.

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Sucrose and the Lipid Environment Modulate Conformational Heterogeneity in the Glutamate Transporter Homologue Glt_{ph}

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Glt_{ph} is a sodium dependent aspartate transporter that is structurally homologous to glutamate transporters; as a result, it provides a model for excitatory amino acid transporters (EAATs) that facilitate glutamate reuptake. Crystal structures suggest a model for transport but do not provide information on biologically relevant intermediate states or effects of the lipid bilayer on the transport cycle. In the present work, distance distributions were measured for Glt_{ph} in three different bilayer systems though site directed spin labelling (SDSL) and double electron-electron resonance (DEER), and membrane depths were measured through SDSL and power saturation to determine if the crystal structures are representative of biologically relevant conformations. Transport domain distances were measured across the subunits of the trimer, and the protective osmolyte sucrose was used to modulate conformational populations. Distance distributions for T375R1 revealed populations with average distances that were close to crystal structure predictions. However, narrowing of the distance distributions and a shift to shorter distances in the presence of substrate suggest that there are biologically relevant states that are not seen in the crystal structures. Sucrose mediated stabilization of the inward facing state suggests increased lipid contacts, and membrane depth measurements showed that T375R1 comes into contact with the lipid environment in the presence of substrate, which suggests a currently unknown role for lipids during the transport cycle. This work was supported by NIGMS, GM035215.

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Design and Characterization of Long and Stable *de novo* Single α-Helix Domains

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Naturally occurring single alpha helical (SAH) domains are unique structural elements displaying high stability across a range of pH and ionic strength conditions. Rich in charged residues (E, K and R), which are thought to form a network of stabilizing ionic interactions, SAH domains play a key role as flexible elements that bridge functional domains in proteins. The best-studied examples of SAH domains come from myosin motor proteins in which they can replace the canonical lever. We recently showed that inner centromere protein (INCENP) has a long (>200 residue) SAH domain. To gain more insight into the properties of SAH domains we designed and characterized 98-residue *de novo* polypeptides with 7-residue repeat patterns, AEEEXXX (X = K or R). The *de novo* polypeptides EK3 (AEEEKKK repeat) and EK2R1 (AEEEKRK) formed highly stable monomeric α-helices *in vitro*, with EK2R1 being more helical and thermally stable than EK3. Surprisingly, ER3 (AEEERRR) and EK1R2 (AEEEKRR) did not, indicating that K and R are not fully interchangeable. Protein Data Bank analyses and molecular dynamics simulations help rationalize these findings: E–R combinations form more salt bridges and are more dynamic than E–K pairings. Precise control of the K:R ratio thus generates helical peptides with distinct properties, which have potential applications in protein engineering and synthetic biology. To demonstrate this we designed and expressed a chimera of myosin-5 HMM with part of its original lever replaced by the artificial SAH EK3. *In vitro* motility assays and TIRF experiments show that the chimera protein retains its ability to bind to and move along actin filaments. Artificial SAH domains mimic the behaviour of natural SAH domains both outside and within the protein context and may be tailored for specific, protein engineering needs.

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Molecular Dynamics Simulations of DNA Pol β Phosphorylation-Induced Structural Changes

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DNA polymerase β is a 39 kDa enzyme that is a major component of Base Excision Repair in human cells. The enzyme comprises two major domains, a 31 kDa domain responsible for the polymerase activity and an 8 kDa domain, which bind ssDNA and has a dRP Lyase activity. The atomic structure for the enzyme has recently been elucidated. DNA polymerase β was shown to be phosphorylated *in vitro* with Protein Kinase C at serines 44 and 55, resulting in loss of its polymerase enzymic activity, but not its ability to bind ss DNA (Tokai *et al*, J Biol Chem. 1991;266(17):10820-4.). In this study, we investigate the potential phosphorylation-induced structural changes for DNA polymerase β using molecular dynamic simulations. Different systems were simulated with the following types of phosphorylations; serine 44, serine 55, and serine 44 and 55 together. The simulations show DNA polymerase β structure was subjected to highest structural deviations (RMSD) and fluctuations (RMSF) with serine 44 phosphorylation. In addition, the structure becomes more swollen as evidenced by higher radius of gyration (Rg) values. Cluster analysis of structures was also performed and confirmed the stronger effect of phosphorylation at serine 44. The results suggest that the phosphorylation of serine 44 is the major contributor to structural fluctuations that lead to loss of enzymatic activity.

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G Protein Signaling in Plants: Characterization of Alpha and Gamma Subunits

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In plants heterotrimeric complexes of G proteins (consisting of alpha, beta, and gamma subunits) regulate several signaling pathways including seed germination, seedling development, organ shape and size determination. The alpha subunit has GTP binding and hydrolysis activity and the beta- gamma subunits interact with downstream effectors as a heterodimer. Some structural homology among the plant and mammalian subunits have led to early assumptions about similarities in the activation and transduction mechanisms in the two systems. However, recent evidence on the lack of membrane receptors in plants, the constitutively active state of the plant alpha subunit and the existence of a large family of gamma subunits indicate that the mechanisms involving the plant proteins may be significantly different from those in their mammalian counterparts.

In our group the alpha subunit from A. thaliana (AtGPA1), an N-terminal mutant (GPA1t), the gamma subunits (AGG1, AGG2), and the rice gamma subunits (RGG1 and RGG2) were expressed in yeast and bacteria. Absorbance spectroscopy, circular dichroism spectropolarimetry, and dynamic light scattering (DLS) analyses show the structural and stability differences between AtGPA1 and GPA1t as well as among all gamma subunits. DLS, native-PAGE and small angle X-ray scattering measurements reveal the stable oligomeric forms of the proteins in solution indicating possible functional roles for the oligomers. Results also demonstrate the high level of the flexibility in the structures of all subunits. Models for possible roles of different subunits in G protein signaling in plants will be presented.
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Spectroscopic and SAXS Studies of Human Prion Protein Variants Complexed with Divalent Cations
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Neurodegenerative diseases are probably the most difficult diseases to find for them a successful treatment strategy. The discovery of new potential drugs, which can be useful in the treatment of neurodegenerative diseases, require full structural characterization of all proteins involved in development of these diseases. One of the human neurodegenerative disorders is Creutzfeldt-Jakob disease (CJD). This disease is caused by misfolded (pathogenic) form of prion protein (PrP), which is a membrane protein exposed into synaptic cleft [1]. So far, the structures of several variants of prion proteins from various organisms (hamster, bovine or human) have been solved by protein crystallography and NMR. The molecule of cellular form of human PrP protein is composed of two domains: unstructured and flexible N-terminal domain containing four tandem octarepeats and structured C-terminal domain [2]. The aim of our study was to obtain the structural information for several complexes of the human prion protein. As an object of the study presented here we have chosen the cellular form of human prion protein PrPC (23-231) and its mutant form (H61A). The low resolution structures of both forms complexed with divalent cations were characterized by SAXS technique. The conformational changes of proteins studied were also detected by spectrofluorimetry, circular dichroism and NMR. This work was supported by the funds from the National Science Centre (Poland) granted on the basis of decision no. No. 2014/15/B/ST4/04839.
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939-Pos Board B7
Ergodicity Measurements in Native Protein Ensembles using Solid-State Nanopores
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Accompanying the diverse palette of functions exhibited by proteins is a rich set of structural properties. For example, whereas a particular protein function such as electron transfer requires well-positioned residues in space to provide precise interatomic distances, many enzymes require conformational flexibility to facilitate substrate recognition and catalysis. While crystallography provides atomic-level structural information, there are no rival techniques that can analyze the size and extent of structural diversity in proteins in their solution environment. We present here evidence that gradient-driven transport of proteins in their native state through nanopores can report on a protein's mean size, structural fluctuations, and conformational changes. Protein transport measurements were made through crystallized hafnium oxide and silicon nitride nanopores using high-bandwidth measurements. First, the sizes of various proteins in solution was estimated from mean fractional current blockade amplitude values, matching values from literature and simulations. Further, we find a good correlation between the widths of fractional current amplitude distributions and amplitudes of protein fluctuations computed from simulations. Finally, we demonstrate the detection of conformational changes in calmodulin, a protein that changes its conformation upon calcium-ion binding.

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Biophysical Studies of TRAIL-Based Anticancer Fusion Protein AD 051.4
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The development of novel therapeutic agents that activate the apoptotic pathways in tumor cells focuses laboratories around the world. Well characterized mechanisms that can result in cellular apoptosis are those induced by the death receptors of the tumor necrosis factor receptor superfamily (DRs) and their respective death ligands (*e.g.* TRAIL). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has no toxic effects on normal cells and induces apoptosis in various tumor cell types. However, some of tumors remain resistant to TRAIL. Thus, it is desirable to find an effective and breaking the resistance TRAIL-derivatives with increased sensitivity and potency. In our work we present recombinant TRAIL ligand protein fused with a peptide corresponding to the exon 6a-encoded domain of vascular endothelial growth factor, named AD 051.4. In our laboratory we did *in vitro* and *in vivo* experiments which confirmed its pro-apoptotic and anti-angiogenic activities and tumors growth regression. AD 051.4 currently enters the preclinical studies as an anticancer agent. Here, we focus on biophysical aspects of the AD 051.4 fusion molecule. We started with the *in silico* simulation of the protein potential structure. Further, with the use of circular dichroism and ion mobility mass spectrometry we checked if our purified protein upheld the folded structure. The protein forms homotrimers as TRAIL, which was showed by size exclusion chromatography coupled with multiangle light scattering. Using two different techniques, surface plasmon resonance and biolayer interferometry, we confirmed the binding abilities with TRAIL receptors. According to our expectations, fusion increased the range of interactions with other partners such as heparin and growth factor receptors. This indicates that AD 051.4 may have greater advantage over the TRAIL effectiveness against tumor cells.

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On the Interaction of Alkyl-Functionalized Ionic Liquids with Model Proteins: A Spectroscopic and Structural Study
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Ionic liquids (ILs) are salts that are liquid at temperatures smaller than 100°C and are gaining prominence in the so-called green chemistry. In order to understand its interaction with biologically relevant systems, we conducted a systematic study of the interaction of three different ILs ([C_{10}mim][Cl], [C_{12}mim][Cl] and [C_{14}mim][Cl]) with three proteins (BSA, HSA and Lysozyme), by means of UV-vis absorption, fluorescence, circular dicrhoism (CD) and small angle X-ray scattering (SAXS). We observed fluorescence quenching of all studied proteins, the decrease were (for BSA, HSA and lysozyme, respectively): (55 ± 3)%, (16.1 ± 0.8)% to (4.1 ± 0.2)% in the presence of 0.6mm [C_{14}mim][Cl], similar trend were obtained for [C_{12}mim][Cl] and [C_{10}mim][Cl]. We also note the shift of the fluorescent peak of BSA and HSA for shorter wavelengths (blue-shift), as the IL content was increased. The maximum shift ($\Delta\lambda$) achieved corresponded to (21 ± 1) nm for both albumins, whereas no significant displacement was observed for lysozyme. SAXS data indicate an increasing in the proteins radius of gyration (Rg) as ILs was added in the solution. Rg of BSA, HSA and lysozyme in the absence of IL are (29 ± 1) Å, (30 ± 1) Å and (15 ± 1) Å, respectively, and go to (46 ± 1) Å, (44 ± 1) Å and (20 ± 1) Å, respectively, in the presence of 0.6mm [C_{14}mim][Cl]. CD data suggest a slight loss of secondary structure of both albumins (BSA and HSA), from 80 to 65% of α-helix in the absence and presence of 0.6mm [C_{14}mim][Cl], respectively. Taking together, our data suggest that the interaction between IL and the proteins is initially driven by electrostatic forces, having also a major hydrophobic contribution. We believe this work provides new information about the interaction of ILs with model proteins, indicating its ability to alter the conformation of the same.

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Charge Transfer Transitions Originating from Charged Amino Acids Account for 300-800 nm UV-Visible Electronic Absorption Spectra in Proteins
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The electronic absorption spectra of the protein folds are primarily characterized over the ultraviolet region (180 nm to 320 nm) of the electromagnetic