Posters

Protein Conformation II

1203-Pos

Molecular Dynamics Simulations Predict A pH-Dependent Conformational Change in the C-Helix of Cell Cycle Checkpoint Kinase Wee1

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Wee1 is the cell cycle checkpoint kinase that phosphorylates Tyr15 of Cdk1, maintaining it in an inactive state. Cdk1 regulates the transition from S to G2/M phases in eukaryotic cells and is activated upon dephosphorylation of residues Thr14 and Tyr15 and cyclin B1 binding. We previously reported that increased intracellular pH (pHi) (> 7.2) promotes G2/M transition. Moreover, we found that in cells having low pHi (< 7.2) the abundance of phosphorylated Cdk1 (pTyr15) is aberrantly sustained. We are testing the hypothesis that Wee1 kinase activity might be pH sensitive, with higher activity at lower pH. The C-helix in Wee1 contains four contiguous residues unique to the Wee1 family centered around His350, which faces the ATP binding site. Based on this observation, we hypothesized that changes in pHi could affect the C-helix conformation and hence catalytic activity (C-helix conformation modulates activity in many other kinases). Molecular dynamics simulations of Wee1 were performed while allowing His350 to be either neutral or charged, capturing the hypothetical protonation state of His350 at high and low pHi, respectively. Most portions of the kinase domain showed only small changes at the end of the two 10 ns simulations. However, when His350 was neutral, the C-helix adopted a conformation that closely mimics the inactive state of other kinases. In contrast, when His350 was charged, the C-helix adopted a conformation similar to the active state of other kinases wherein the C-helix was rotated towards the active site. These data suggest that pH-sensitivity in Wee1 may be mediated by the influence of the protonation state of His350 on the C-helix conformation. We are currently testing these predictions using NMR and biochemical approaches.

1204-Pos

The L49F Mutation in Erythroid Alpha Spectrin Induces Local Disorder in the Tetramer Association Region: Fluorescence And Molecular Dynamics Studies of Free and Bound Alpha Spectrin

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The bundling of the N-terminal, partial domain helix (Helix C') of human erythroid α-spectrin (αI) with the C-terminal, partial domain helices (Helices A' and B') of erythroid β-spectrin (βI) to give a pseudo spectrin structural domain (triple helical bundle A'B'C') has long been recognized as a crucial step in forming functional spectrin tetramers in erythrocytes. We have used fluorescence studies to obtain apparent polarity and Stern-Volmer quenching constants of Helix C' of αI bound to Helices A' and B' of βI. These properties were used to guide us in homology modeling with a previous NMR structure as the template. The homology models then became input structures for molecular dynamics simulations for both wild type (WT) and an αI clinical mutant Spectrin Lyon (L49F). The simulation output structures show a stable helical bundle for WT, but not for L49F. In WT A'B'C', four critical interactions were identified: two hydrophobic clusters and two salt bridges. However, in L49F, the C-terminal region of Helix C' was unable to assume a helical conformation and one critical hydrophobic cluster was disrupted. Other molecular interactions critical to the WT helical bundle were also weakened in L49F. We suggest that these conformational changes lead to a lower tetramer levels observed in Spectrin Lyon patients.

1205-Pos

Characterization of Conformational Transitions in Src Kinase using the String Method with Swarms-of-Trajectories and Markovian Milestoning

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Src tyrosine kinases are a family of signaling proteins playing key roles in intracellular signaling pathways. Dysregulation of Src activity has been implicated in several types of cancer. An important conformational change in Src involves the highly conserved DFG motif (Asp404, Phe405, Gly406) adjacent to kinase ATP-binding site. In the majority of available crystal structures, the DFG points into the ATP-binding site where it coordinates a magnesium ion (DFG-in conformation). A recent X-ray structure shows that c-Src is also able to adopt a DFG-out conformation, in which the DFG motif is flipped by approximately 180°. Understanding the factors controlling the flipping of the DFG motif is important for designing highly selective kinase inhibitors. We first determine atomistic pathway of DFG in-to-out transition using the string method with swarms-of-trajectories with all-atom molecular dynamics (MD) simulations. Images along the pathway are then used as centroids to construct a complete Voronoy tessellation in the collective variables space, which then serve as a ''basis set'' to define the discrete micro-states and to build a reduced stochastic model. MD trajectories with ''soft-wall'' restraining hyper-plane potentials are launched to keep a system inside each Voronoy cell. By monitoring the wall-to-wall transitions among the walls separating the Voronoy cells, we estimate the free energy and mean first passage time (MFPT) of DFG flipping. An improved understanding of the interplay of the structure, dynamics and activity of Src kinase will help the development of new inhibitors for targeted cancer therapy. [Supported by NIH grant CA093577]

1206-Pos

Probing the Structure of Membrane Proteins Using Pulsed EPR ESEEM Spectroscopy

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Current methods used for determining the secondary structure of membrane proteins require large sample sizes and have long data acquisition times. Therefore using pulsed EPR spectroscopic technique of Electron Spin Echo Envelope Modulation (ESEEM) is advantageous due to the requirement of less sample, short data acquisition, and high sensitivity. Using this technique, we can determine short to medium range distances (up to 8 Å) between a site-specific nitroxide spin label (MTSL) and a nearby NMR-active isotopic labeled residue for a variety of peptides and proteins which ultimately determine the difference between an α-helical and β-sheet secondary structure. The information can be obtained using a three-pulse ESEEM sequence which allows for the detection of the fundamental nuclear spin transitions. It is possible to calculate the radial distance between a paramagnetic electron and a weakly coupled nucleus because the modulation depth produced by a weakly dipolar-coupled nucleus is inversely proportional to the radial distance. This new method is applied to two different membrane peptides, M2δ AChR, and KIGAKI, which have α-helical and β-sheet structural components respectively. A nitroxide spin probe is attached to a specific Cys residue at a given position *I,* and a ^{2}H isotopic labeled residue is attached to a nearby residue ($i + 3$). The corresponding data shows that an α-helical structure will yield a very large ^{2}H peak, whereas a β-sheet will yield a much smaller ^{2}H peak, thus a difference can be observed between the two secondary structures. The ESEEM technique requires less protein sample (about 75 μg) and data acquisition only takes about 1 hour which makes it a simple, powerful, and effective technique in the determination of the secondary structure of a membrane protein.

1207-Pos

SDSL-EPR Study of a C-terminal Segment of Myelin Basic Protein in a Myelin Mimetic Environment

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Myelin basic protein (MBP) is a peripheral membrane protein and a major component of human central nervous system myelin. It is a multifunctional, highly positively charged, intrinsically disordered protein that undergoes extensive post-translational modifications. Its multifunctionality includes among others, binding of cytoskeletal proteins to a membrane surface and polymerization and bundling of cytoskeletal proteins. These interactions are modulated by interaction of MBP with Ca^{2+}-calmodulin. We report here the use of site-directed spin labeling and continuous wave power saturation electron paramagnetic resonance spectroscopy (SDSL-EPR) to examine the conformation of segment A141-L154 of a recombinant 18.5 kDa murine isoform of MBP in a reconstituted membrane environment. This segment overlaps the primary calmodulin binding region (T147-D158) of MBP and is predicted, on the basis of helical wheel constructs, to form an amphipathic alpha helix. Solution NMR investigations also showed this region to exist as a transient alpha helix in 30% TFE. Our measurements using SDSL-EPR reveal that this region forms an extended helix with a period of ~3.8 residues per turn and with a tilt angle of ~4.3 degrees with respect to the plane of the lipid bilayer. The N-terminal end of the helix appears to be buried more deeply in the membrane bilayer than the C-terminal end. The C-terminal region of the segment bears two Lys residues whose side chains interact with the lipid head groups causing this end to be more exposed. The accessibility of the C-terminal region of A141-L154, which forms a part of the primary calmodulin binding segment, could facilitate interaction of this region

with cytosolic proteins viz., calmodulin and modulate cytoskeletal binding to the oligodendrocyte plasma membrane.
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1208-Pos
A Study of the Conformation of HIV Nef Bound to Lipid Membranes by Neutron Reflectivity
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Nef is one of six HIV-1 accessory proteins and directly contributes to AIDS progression. Nef associates with membranes and may require a transition from a solution conformation to a membrane-associated conformation. It has been hypothesized that a transition from a ''closed'' conformational form to an ''open'' form enables interaction of Nef with cellular proteins. Despite its obvious disease importance, there is little or no direct information about the conformation of membrane-bound Nef. In this work we used neutron reflection to reveal details of the conformation of membrane-bound Nef. Nef was bound through an N-terminal His tag to Langmuir monolayers of DPPC mixed with a synthetic metal-chelating lipid. Several methods were found to achieve a dense monomolecular layer of membrane-bound Nef, despite its tendency to form oligomers at high concentration. At the conditions of this initial study (65% DSIDA 35 mol% DPPC, 20 mM Tris, 20 C, pH 8.2), for the large majority of the bound population the core domain of membrane-bound Nef lies within a few Å of the lipid headgroups. The N-terminal arm is directly against the lipid headgroups with a small portion inserted. The results also indicate that the disordered loop extends from the core domain into the solution. The data also suggest that for a very small fraction of the bound population the N-terminal arm extends normal to the membrane and the core domain is displaced ~50 Å from the membrane. Some ramifications of these results for the activity of Nef are discussed.

1209-Pos
Site-Directed Spin Labeling Electron Paramagnetic Resonance Studies of Flap Conformations and Flexibility in Multiple HIV-1 Protease Variants
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Human immunodeficiency virus type 1 protease (HIV-1PR) is a 99 amino acid homodimeric aspartic protease that plays an essential role in the maturation and life cycle of the retrovirus HIV-1, as it functions in regulating post-translational processing of viral polyproteins gag and gag-pol. Accessibility of substrate to the active site is mediated by the conformational changes within two β-hairpins, or flaps. Site-directed spin labeling (SDSL) in conjunction with continuous wave and pulsed electron paramagnetic resonance spectroscopy was used to monitor the conformations of the flaps in HIV-1PR. Six inactive (D25N) HIV-1PR constructs were purified and spin labels were incorporated into the flaps at the aqueous exposed sites K55 and K55'. Constructs included Subtypes B, C, F, circulating recombinant form CRF01_A/E, and patient isolates V6 and MDR 769. For all constructs, two naturally occurring cysteine residues (C67 and C95) were substituted to alanine residues to allow for site-specific labeling as well as to avoid intramolecular disulfide cross-linking. B, F, C, and CRF01_A/E constructs contained three stabilizing mutations that provide protection from autocatalytic cleavage: Q7K, L33I, and L63I. Pulsed EPR results have shown that sequence variations within the subtypes of HIV-1 protease alter the average flap conformations within the apo-enzymes. With detailed data and error analysis, these altered distance profiles can be understood as shifts in the conformational sampling of four distinct HIV-1PR conformations, with some states having enhanced flexibility or structural instability, which may play an important role in viral fitness and drug-resistance. Additionally, DEER data was collected on each construct in the presence of nine different FDA-approved protease inhibitors and a non-hydrolysable substrate mimic called Ca-P2. Continuous wave EPR was used to monitor the autoproteolysis of active (D25) Subtype F and CRF01_A/E constructs.

1210-Pos
Correlated Force-Optical Spectroscopy of Single Human Nucleolar Phosphoprotein-140 Immobilized on Nano-Array Patterns
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Human nucleolar phosphoprotein-140 (hNopp140), an intrinsically unstructured protein (IUP), is known for its regulatory behavior of uncontrollable cell growth like in cancer and its unusual structural characteristics of a high percentage of flexible regions or extended loops. It is also one of the most highly phosphorylated mammalian proteins, of which interactions with ligands and other proteins depend strongly on the degree of phosphorylation. In this work, we employed a correlated force-fluorescence imaging and spectroscopic technique in conjunction with a nano-array patterning technique, to investigate the intrinsic shape of hNopp140 and its structural changes by phosphorylation, ligand binding and interactions with other proteins. For this, we prepared nano-array patterned surfaces containing hexagonally well-separated spots (~300 nm in diameter, ~ 2 um in distance), functionalized the spots with specific covalent linkers for targeting the cys residue of hNopp140, and immobilized single hNopp140 covalently on each spots. The presence of single hNopp140 on each spot was confirmed by single molecule fluorescence behavior of fluorescently labeled hNopp140 in TIRF mode and by subsequent high resolution AFM imaging. We finally performed single molecule force-fluorescence spectroscopy of hNopp140 itself and its complexes with ligands such as doxorubicin, mitoxantrone, and proteins such as CK2 under the precisely controlled nano-environments, and attempted to reveal the structural nature of hNopp140 and its intra- and inter-molecular interactions at single molecular level.

1211-Pos
Allosteric Inhibition of Thermus Thermophilus Phosphofructokinase
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Thermus thermophilus phosphofructokinase (TtPFK) comes from an extreme thermophile and exhibits entropically-driven inhibition by phosphoenolpyruvate (PEP). Interestingly, a PFK from the moderate thermophile Bacillus stearothermophilus also exhibits entropically-driven inhibition, while enthalphically-driven inhibition is observed for PFK from mesophilic E. coli. Although the thermodynamics of inhibition are similar for TtPFK and BsPFK, TtPFK exhibits a much weaker coupling between the inhibitor and substrate ($\Delta Gay = 1.60 \pm 0.04$ kcal/mol) when compared to that of BsPFK ($\Delta Gay = 5.0 \pm 0.9$ kcal/mol). Sequence alignment and crystal structures of BsPFK suggest that there is a network of interacting residues leading from the allosteric binding site to the active site. In the apo form, H215 forms a hydrogen bond with T158 and T156 interacts with D12 across the interface. In the inhibitor-bound form, T158 forms a hydrogen bond with D12. In TtPFK these interactions are missing due to substitutions at positions 215 (Ser) and 158 (Ala). Changing the amino acid residues at these positions to the corresponding amino acids in BsPFK resulted in an increase in coupling free energy to $\Delta Gay = 2.47 \pm 0.02$ kcal/mol for S215H and $\Delta Gay = 2.45 \pm 0.04$ kcal/mol for A158T. Currently the double mutant A158T/S215H is being investigated to see if the coupling can be augmented to the level of BsPFK coupling. Supported by NIH grant GM33216 and Welch Foundation grant A1548.

1212-Pos
Nucleotide Binding Induces Conformational Changes in Full-Length ClC-5
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Mutations within the Cl^-/H^+ transporter ClC-5 lead to Dent's disease, a kidney condition characterized by proteinuria. Numerous disease-causing mutations in ClC-5 translate into truncations of the carboxy terminus (Ct), a region binding adenine nucleotides and mediating ATP-dependent regulation of ClC-5 activity [Meyer, S. *et al.,* 2007; Zifarelli, G. & Pusch, M., 2009]. As the mechanism underlying allosteric regulation of ClC-5 activity is unknown the major goal of this work was to capture dynamic nucleotide-dependent conformational changes in ClC-5 Ct. Sedimentation velocity on purified Ct revealed a more compact peptide with ATP bound evident by an increase in its sedimentation coefficient (1.18 to 1.23 $s_{20,\omega}$) and a decrease in its frictional coefficient ratio (1.55 to 1.46) with ATP present. Photo-affinity labelling of ClC-5 in crude membranes with the analog γ^{32}P-ATP-γbenzophenone ensured full-length protein directly bound ATP. As the intracellular Ct is tethered to the membrane domain through the R helix, conformational changes in response to ATP binding could evoke global structural changes in ClC-5. In support of this hypothesis, ATP binding mediated global conformational changes in membrane bound ClC-5 as revealed by ATP-dependent increases in the accessibility of endogenous cysteine residues (2.04 ± 0.34 times the control). Importantly, changes in accessibility were not observed in D727A ClC-5, the mutant unable to specifically bind ATP in photo-labelling experiments. Future work will focus on uncovering the physiological significance of nucleotide-dependent regulation of ClC-5 activity in endocytic uptake and acidification in the kidney. This work was funded by a Kidney Foundation of Canada operating grant to CB and a studentship from NSERC to LW.