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Conformational Dynamics of Short C-13-Labeled Helical Peptides as Measured by Dynamic NMR Spectroscopy

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Changes in the Molar Ellipticities of HEWL Observed by Circular Dichroism and Quantitated by Time Resolved Fluorescence Anisotropy Under Crystallizing Conditions.

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Fluid models for simple colloids predict that as the protein concentration is increased, crystallization should occur at some sufficiently high concentration regardless of the strength of attraction. However, empirical measurements do not fully support this assertion. Measurements of the second virial coefficient (B_{22}) indicate that protein crystallization occurs only over a discrete range of solution parameters. Furthermore, observations of a strong correlation between protein solubility and B_{22}, has led to an ongoing debate regarding the relationship between the two. Experimental work in our lab, using Hen Egg White Lysozyme (HEWL), previously revealed that the rotational anisotropy of the protein under crystallizing conditions changes systematically with pH, ionic strength and temperature. These observations are now supported by recent work revealing that small changes in the molar ellipticity also occur systematically with changes in ionic strength and temperature. This work demonstrates that under crystallization conditions, the protein native state is characterized by a conformational heterogeneity that may prove fundamental to the relationship between protein crystallization and protein solubility.

2452-Pos Board # B50

Conformational Dynamics of Short ^{13}C-labeled Helical Peptides as Measured by Dynamic NMR Spectroscopy

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The helix is the most common secondary structural motif in proteins, and it continues to attract much theoretical and experimental interest. We are using oligomers of the strongly helix forming residue α-aminoisobutyric acid (AIB) as models of helical structure and dynamics. Oligomers of this achiral residue are known to form left- and right-handed 3_{10} helices. The rates of interconversion between enantiomeric left- and right-handed helices can be measured using dynamic NMR techniques (Hummel et al., *Angew. Chem. Int. Ed.* **26**, 1150 (1987)). We have synthesized hexameric and octameric oligomers of AIB, each ^{13}C-labeled at a single residue. Using lineshape analysis of the labeled ^{13}C residues, we have measured the enantiomerization kinetics for these organic-soluble peptides in CD_2Cl_2. Within the assumption of a fully cooperative enantiomerization transition, the octamer and hexamer show a similar $\Delta H^{\neq}$ of around 30 kJ/mol. However, the octamer has a larger, more negative $\Delta S^{\neq}$ than the hexamer. The thermodynamic activation parameters for the conformational enantiomerization dynamics of these peptides will be discussed.

2453-Pos Board # B51

Homology Modeling of Myson Light Chain Kinase for Structure Based Drug Design.

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Myosin Light Chain Kinase (MLCK) is a regulatory enzyme in smooth and skeletal muscle contraction. MLCK has been shown to be involved in invasion and metastasis of pancreatic cancer and glioma along with bronchial diseases. Identification of specific inhibitors may offer a therapeutic strategy for preventing the proliferation of certain cancers and diseases. In this study, we have built a model of the smooth and skeletal MLCK's catalytic core and regulatory domain based on recently available crystal structures of the Death-associated Protein Kinase (DAPK), Twitchen Kinase and Titan using the Accelrys, Inc. software Insight II HOMOLOGY. The percent identity between the catalytic cores of these enzymes and smooth and skeletal MLCK is a minimum of 47% as opposed to 31% with the cAMP-dependent protein kinase catalytic core used for modeling of smooth MLCK by Knighton et.al (1992). We believe that the new model is more reliable and a more accurate representation of MLCK and therefore can be used for structure-based design of specific smooth and skeletal MLCK inhibitors. In addition, the new models offer new strategies for further experiments that will give insight into the molecular bases of substrate specificity between smooth and skeletal MLCK.

2454-Pos Board # B52

A highly conserved arginine is critical for the functional folding of Inhibitor of Apoptosis (IAP) BIR domains

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The Inhibitor of Apoptosis (IAP) proteins are found in all animals and regulate apoptosis (programmed cell death) by binding and inhibiting caspase proteases. This inhibition is overcome by several apoptosis stimulators, including *Drosophila* Hid and mammalian Smac/DIABLO, which bind to 65 residue Baculovirus IAP Repeat (BIR) domains found in 1-3 copies in all IAPs. Virtually all BIRs contain 3 Cys and a His that bind zinc, a Gly in a tight turn, and an Arg. The functional and structural role of the Arg was investigated in isolated BIR domains from the baculovirus *Orgyia pseudotsugata* Op-IAP and the *Drosophila* DIAP1 proteins. Mutation of the Arg to either Ala or Lys abolished Hid and Smac binding to BIRs, despite the Hid/Smac binding site being located on the opposite side of the BIR domain from the Arg. The mutant BIR domains also exhibited weakened zinc binding, increased sensitivity to limited proteolysis, and altered circular dichroism spectra indicative of perturbed domain folding. Examination of known BIR structures indicates that the Arg side chain makes simultaneous bridging hydrogen bonds and a cation-π interaction for which the Arg guanidino group is uniquely well-suited. These interactions are likely critical for stabilizing the tertiary fold of BIR domains in all IAPs, explaining the conservation of this residue.

2455-Pos Board # B53

Multi-resolution modeling of protein dynamics-function relationship

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Protein's function closely correlates with its dynamic behavior. We have used both the atomic detailed molecular dynamics and the residue level conformation analysis to study protein dynamics near its native conformation. In protein-protein interaction study, we have found the residues involved in high-frequency mode correlate with the its binding sites. This information can then be used in protein binding site prediction and protein interaction network prediciton. In force-induced unfolding of mechanical proteins, the low frequency dynamic modes of the protein dramatically changed during the key unfolding events.

2456-Pos Board # B54

Investigation of cooperative phase transition in elastin aqueous solution

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Elastin has been widely investigated for its unique properties in solution. Aqueous solutions of α-elastin, a fragmentation product from elastin fiber, undergo an inverse phase transition upon temperature increase. In this presentation, the phase transition of