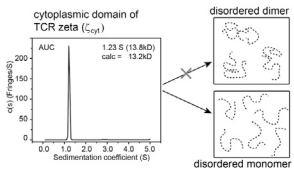
transient intra- rather than intermolecular interactions. Furthermore, even disulfide crosslinking of ζ_{cyt} N-termini, in a configuration reminiscent of T cell receptor clustering, fails to lead to an association of protomers. SEC-MALS confirms the monomeric state of ζ_{cyt} but reveals a curious concentration-dependent shift of the elution volume of ζ_{cyt} that may previously have been interpreted as dimerization. Our data show that ζ_{cyt} does not form a highly disordered protein complex but leave open the question as to whether completely disordered dimers or other oligomers exist in nature.



3480-Pos Board B208

Mapping Residual Structure in Disordered Protein Ensembles with Millisecond H/D Exchange Mass Spectrometry

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Interactions of intrinsically disordered proteins (IDP) with their binding partners often involve coupled binding and folding. A long-standing question is the extent to which folding of the IDP is mediated by selection of a folded conformer from the disordered state ensemble rather than folding induced by interaction with the binding partner. Answering this question requires detailed information about the disordered state ensemble, in particular the extent to which the IDP possesses residual structure. Yet obtaining this type of information at near-atomic resolution remains challenging. To address this need, we have developed an approach based on millisecond quench-flow amide H/D exchange and mass spectrometry to measure residual structure. In the present work, we examine residual structure in the disordered CBP-binding domain of ACTR as a model system for validation. Following millisecond H/D exchange and acid quench, digestion with pepsin produced a set of 67 highly-overlapping fragments covering the entire 77-residue sequence. Residue-by-residue analysis of empirically-determined H/D exchange half-life obtained from each ACTR fragment provided exchange kinetics at near-residue resolution.

In ACTR, we found that the regions that are known adopt an α-helical fold upon binding to CBP became more protected from H/D exchange than the structured loop regions. We also found that most of the N-terminal region, which does not appear in the solved structure, was the least protected. There was also evidence of slight protection in a short stretch of the N-terminal region. Our results are consistent both with a recent analysis of residual structure obtained from NMR secondary shift measurements and with the AGADIR helicity prediction algorithm. Our results demonstrate the utility of millisecond H/D exchange for mapping secondary structural propensity in disordered state ensembles with near-residue resolution.

3481-Pos Board B209

Structure and Internal Dynamics of Calcitonin Family Peptides: Implications for Amyloid Formation

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The calcitonin peptide (Ct) family comprises the intrinsically disordered proteins amylin (IAPP), calcitonin gene-related peptide (CGRP), calcitonin, and adrenomedullins. These are genetically and structurally related hormone peptides that are able to bind to each other's receptors, though with varying degrees of affinity. Some of these peptides form amyloid fibers, while others do not. They contain highly conserved sequence elements that have been experimentally shown to affect the secondary structural preferences of these peptides. The effect of such conserved elements on tertiary structure has not been experimentally explored to the same extent. Detecting tertiary structural properties of IDPs is considerably more challenging due to fast reconfigurations of the backbone over a wide range of possible conformations. High resolution time-resolved techniques are needed. We use a nanosecond laser spectroscopy technique to measure transient tertiary contact formation. This technique reveals information on the structure and internal dynamics of IDPs. We compare members of the Ct family which differ in hydrophobicity and net charge, and study the effect of proline mutations on contact formation rates. We find that functionally required, conserved sequence elements play an important role in determining the structure, internal dynamics and aggregation properties of these peptides.

3482-Pos Board B210

Enantiospecific Recognition of the Intrinsically Disordered C-Myc Oncoprotein by Small Molecules

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The prevalence of intrinsically disordered proteins (IDPs) in cell signaling and disease makes them attractive targets. Despite the absence of defined tertiary structure, small molecules can bind IDPs at sites determined by a short, linear segment of the protein's primary sequence. The oncoprotein c-Myc, a transcription factor that must undergo coupled folding and binding to its obligate partner Max in order to interact with DNA, is an attractive system for understanding specificity in small molecule binding to IDPs. Three independent small molecule binding sites exist in the bHLHZip region of c-Myc, the segment necessary for coupled folding and binding to Max. The chiral small molecule 10074-A4 recognizes one of these sites. A racemic mixture of 10074-A4 exhibits a circular dichroism signal upon binding to c-Myc indicating an enatioselective interaction of the molecule with the protein. We provide a model for this induced circular dichroism signal based on conformational selection in the binding enantiomer, and we synthesize the pure enantiomers and compare their binding. Derivatives of 10074-A4 were synthesized and these also display enatiospecific binding. Even though c-Myc is disordered, and remains so in the complex, it specifically recognizes both the chemical functionalities in the small molecule and their particular three dimensional arrangement.

3483-Pos Board B211

Characterization of the Intrinsically Disordered Region of the Soluble Guanylate Cyclase Alpha-1 Subunit

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Soluble guanylate cyclase (sGC) is a heterodimeric protein, which is activated by nitric oxide, stimulating the conversion of guanosine triphosphate (GTP) to guanosine monophosphate (cGMP). Decreased sGC activity is linked to atherosclerosis, aging, loss of memory, acute ischemia, while increased sGC catalytic activity is associated with endothelial cell proliferation, vasodilation, cell motility and survival. The activation, catalytic activity and structure of sGC are widely studied, yet the mechanism behind sGC regulation is not well understood. Soluble guanylate cyclase contains four distinct functional domains: the H-NOX, Per Arnt Sim, coiled-coiled and C-terminal catalytic domains. The H-NOX domain of the alpha-1 subunit also contains an intrinsically disordered region (IDR) whose role is not understood since it does not affect the dimerization or catalytic activity of the enzyme. Intrinsically disordered regions are regions that have no defined structure but retain function in a highly flexible state. These regions often undergo conformational changes upon protein-protein interactions. The human sGC IDR is predicted to be the first 69 residues of the alpha-1 subunit's amino terminus but a similar region is not found on the beta-1 subunit. The goal of this study is to characterize the secondary structure of this IDR and probe for protein-protein interaction. The sGC IDR is predicted to contain about 26% alpha helical characteristics utilizing secondary structure prediction algorithms. Circular dichroism studies on the sGC IDR have shown that this region contains a small amount of stable helical structure. It also contains transient helical structure that can be stabilized by 2,2,2-trifluoroethanol. Yeast two-hybrid studies are ongoing to identify interacting partners.

3484-Pos Board B212

The Structural and Kinetic Ensemble of ASB9's N-Terminus and It's Role in Substrate Recognition

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Intrinsically disordered proteins (IDPs) have structural ensembles which lack stable tertiary and secondary structure under physiological conditions. IDPs have remolded the structure-function paradigm, often participating in one-to-many and many-to-one protein interactions. One class of IDPs which have remained largely unstudied are the ankyrin repeat and SOCS (supressor of cytokine signaling) box containing proteins (ASBs). This class of proteins have an intrinsically disordered N-terminus, a six ankyrin repeat domain (ARD), and a C-terminal SOCs box domain. The SOCS box of ASB9 is