p53 interact with SPOP. AUC supports a binding model in which the MATH domain can interact with three binding motifs on p53-90 with macroscopic weak affinities while FA allows the determination of microscopic affinities. In vitro ubiquitination assays and protein stability studies in mammalian cells with a series of G13 SBC motif mutants are used for functional interpretation of our biophysical data. Each SBC motif contributes to SPOP binding in agreement with their similar weak affinities. These results will allow the distinction between a dynamic complex, a static multivalent binding mechanism and large oligomeric assemblies in which many G13s co-operate to recruit multiple SPOPs. The presence of multiple dispersed SBC motifs suggests their contribution to spatial and temporal control of G13 levels in response to Hh signaling.

1204-Pos Board B96
Characterization of the Regulation Mechanism of Protein Kinase CK2 by IP6 and Nopp140, an Intrinsically Disordered Protein

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Protein kinase CK2 is a ubiquitous kinase that can phosphorylate more than hundreds of cellular proteins, and has important roles in cell growth and development. The interaction of the catalytic subunit of CK2 (CK2alpha) with inositol hexakisphosphate (IP6) and an intrinsically disordered protein, Nopp140, has been analyzed to elucidate the IP6 and Nopp140-dependent regulation mechanism of CK2. X-ray crystallography analysis of the complex of CK2alpha and IP6 showed that lysine rich domain of CK2alpha which locates near the active site was important for the binding to IP6. One of the interaction site of Nopp140 to CK2alpha was identified at the amino acid residues 560-580 by measuring the interactions between the peptides representing different regions of Nopp140. Particularly, the phosphorylation at Ser568 of Nopp140 significantly enhanced its interaction with CK2alpha. These results suggested a regulatory model of Nopp140 and IP6 on CK2alpha in which CK2alpha activity is inhibited by Nopp140 and re-activated by IP6 by competitive binding at the substrate recognition site of CK2alpha.

1205-Pos Board B97
Biophysical and Structural Study of Intrinsically Disordered Protein (IDP), Nopp140

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Human Nopp140 is a highly phosphorylated nucleolar protein and involved in the biogenesis of the nucleolus. It interacts with a variety of proteins related to the synthesis and assembly of the ribosome including a ubiquitous protein kinase CK2 which mediates cell growth and prevents apoptosis. We showed that hNopp140 is a highly intrinsically disordered protein (IDP) lacking stable secondary structures over its entire sequence of 710 residues. In this work, we employed various biophysical approach, for example, Circular Dichroism (CD), Electron Paramagnetic Resonance (EPR) and Förster resonance energy transfer (FRET), to investigate the intrinsic nature of hNopp140 and its structural changes by various conditions, interaction with CK2alpha.

1206-Pos Board B98
Structural Studies of Cytoplasmic P53 Interactions

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Besides its well-known transcriptional regulatory functions, the tumor suppressor p53 exhibits pro-apoptotic activities in the cytoplasm that are less completely understood. We are undertaking structural and biophysical characterization of protein-protein interactions that mediate the cytoplasmic function of p53 as a direct activator of apoptosis. Our findings to date have elucidated several mechanistic aspects of this apoptotic pathway. Our detailed descriptions of protein-protein interactions within this pathway may enable the alteration of the cytosolic activity of p53 by mutagenesis and, ultimately, chemical intervention. Pharmaceutical approaches aimed at restoring the transcriptional function of p53 are currently being explored. However, these studies generally do not take account of the cytoplasmic roles of p53. Our studies provide a basis for understanding how p53-targeted small molecules may, through their effects on cytoplasmic p53, alter cellular outcomes.

1207-Pos Board B99
Probing the Protein Folding Code through the Polymer Properties of Low-Complexity Sequences

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Converting primary structure into the tertiary structure of a protein remains an unsolved problem. Many proteins with highly divergent amino acid sequences have similar structures. Given the redundancy of the folding code, distinguishing signal from noise is an inherent problem. Rather than using the sequences of structurally characterized proteins to discern the code, we have adopted a strategy of first understanding the behavior of low complexity sequences using thermodynamic, kinetic and computational methods. We have inserted homopolymers of alanine, glycine and glutamine ranging from 6 to 30 residues into the N-terminus of yeast iso-1-cytochrome c. To maintain solubility, every sixth residue is lysine. Homopolymers of these amino acids are important because long repeats of these amino acids are often associated with Human disease. The homopolymers are inserted between a unique histidine and the heme allowing measurement of the thermodynamics and kinetics of His-heme loop formation under denaturing conditions ranging from 1.5 to 6 M guanidine hydrochloride (GdnHCl). Kinetic and thermodynamic loop formation data show that these homopolymers adhere closely to the expected linear dependence of loop formation probability on the logarithm of loop size predicted by the Jacobson-Stockmayer equation. Thus, the scaling exponent, , and Flory’s Characteristic ratio, , are readily evaluated. In 6 M GdnHCl, all three homopolymers have similar values, but at lower GdnHCl concentrations, decreases for the Glycine-rich sequences relative to the Alanine-rich and Glutamine-rich sequences. Evaluation of shows that chain stiffness follows the order: Glutamine-rich > Alanine-rich > Glycine-rich. We have also shown that insertion of aromatic amino acids into the Alanine-rich sequences significantly stabilizes His-heme loops, providing direct evidence that aromatic residues are structure-forming amino acids critical for biasing the conformational landscape of a polypeptide.

1208-Pos Board B100
Multiple Phosphorylation Sites in Disordered Regions of Ash1 form a Dynamic Complex with Cdc4

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Interactions involving intrinsically disordered proteins (IDPs) are crucial to a wide range of biological signaling and regulatory processes. Many of these interactions are characterized by a disorder-to-order transition upon binding. However, a new class of “fuzzy complexes” have emerged in which the IDP remains largely disordered. Such complexes are characterized by an IDP containing multiple weak binding motifs which individually interact with a binding partner in a dynamic equilibrium. The interaction of phosphorylated Ash1, which participates in the regulation of mating type switching in yeast, with Cdc4 phospho degrons (CPDs) with Cdc4. Ash1 is thought to behave similarly to a cyclin dependent kinase inhibitor Sic1, which requires a threshold level of phosphorylation to overcome electrostatic repulsion towards Cdc4. The polyelectrostatic interaction model implies that electrostatic averaging between rapidly exchanging bound states allows Cdc4 to sense the net charge of its disordered binding partner, which is modulated by phosphorylation. In order to probe the interaction and test the polyelectrostatic model, we use NMR spectroscopy, small angle x-ray scattering and fluorescence anisotropy binding experiments. All CPDs contained in Ash1 420-500 engage Cdc4, one at a time. SAXS indicates that the peptide is relatively compact. Fluorescence anisotropy measurements show the affinities of the entire Ash1 420-500 peptide and individual CPD motifs for Cdc4. These preliminary data are suggestive of a “fuzzy complex” and we are in the process of studying the phosphorylation dependence. The dynamic interaction suggests that Ash1 degradation must occur in an all-or-nothing fashion for biological function.