are functionally relevant. We have shown previously that the intrinsically disordered cyclin dependent kinase (CDK) inhibitor Sic1 interacts with the substrate adapter, Cdc4, of its ubiquitin ligase via multiple phosphorylated binding motifs in a dynamic complex. Cdc4 is the substrate recognition subunit of a culin ubiquitin ligase and targets Sic1 for degradation at the G1/S phase transition of the yeast cell cycle. Individual binding motifs in Sic1 are ordered transiently without a global disorder-to-order transition upon binding Cdc4. The dynamic complex allows for engagement of several phosphorylation sites in a dynamic interface resulting in an affinity that depends on the number of phosphorylation sites in an ultrasensitive manner. The dynamic nature of the complex allows for 'counting' of phosphorylation sites via largely electrostatic interactions. The Sic1-Cdc4 interaction therefore acts as a sensor of the concentration of active kinase and the cell cycle status. We continue to use NMR spectroscopy and other biophysical methods to study dynamic interactions in the ubiquitin proteasome pathway with the objective of unraveling nature's repertoire of disorder in protein function. The combination of disorder with multisite phosphorylation may serve as a general means to set thresholds in regulated protein-protein interactions.

2821-Plat
Intrinsic Disorder in the Basic Regions of bZIP Transcription Factors: What it Means to Be Disordered and Why it Might Matter!
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Intrinsically disordered proteins constitute roughly 30% of the eukaryotic preteome and these include a majority of transcription factors. Our recent work has identified certain organizing principles that have yielded predictive phase diagrams of IDPs. We put these predictions to the test through quantitative studies of disorder in bZIP transcription factors. The results suggest important and novel insights on the evolution of disorder and its implications for DNA binding.

2822-Plat
Intrinsically Disordered Proteins Evolve Differently from Ordered (Structured) Proteins
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There are important differences between the evolution of disordered and ordered proteins. These differences include the types of acceptable amino acid substitutions and the rates at which those substitutions occur. In general, substitutions for most amino acid types occur more rapidly in disordered versus ordered proteins, and this behavior is attributed to relaxed purifying and positive selection. The lack of sequence conservation in disordered regions does not mean that these sequences are functionally unimportant as evidenced by the frequent conservation of characterized or predicted disorder in various functional domains. Additionally, the pattern of amino acid substitutions observed for disordered proteins provides important functional clues. In particular, the least frequent amino acid types in disordered proteins are the most conserved and this conservation is correlated with burial at the interfaces with interaction partners. There are also distinct compositional and structural differences between disordered and ordered proteins. These differences were observed in the initial investigations of disordered proteins, but refining developments are leading to a deeper understanding of the structure and dynamics of disordered proteins. These recent studies show that disordered proteins have sequence-dependent conformational features ranging from extended random coil to collapsed random coil to molten globule, lending support to the concept of a structural continuum connecting disordered and ordered proteins.

2823-Plat
Negative Design in Protein Coils
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The classic folding paradigm, established by Anfinsen and others, has been interpreted to mean that under folding conditions, the native fold is selected from an astronomical number of conceivable alternatives by the constellation of favorable interactions among its amino acid sidechains. This plausible idea is entirely consistent with the characteristic close-packing seen in protein crystal structures, where it is apparent that residues distant in sequence are brought together in space, presumably providing both topological specificity and structural stability. Accordingly, organizing interactions are believed to persist into the native state. This supposition is the basis for knowledge-based potentials, Go models, and the like. Contrary to this view, we present evidence from simulations coupled with an analysis of the protein coil library (http://www.roselab.jhu.edu/coil/) that the overall fold is established prior to eventual sidechain close-packing. In this process, chain organization depends not only on selecting favorable interactions but also on rejecting unfavorable ones. Eliminating those interactions that result in steric clashes or unsatisfied hydrogen bonds winnows fold space substantially. Accordingly, such interactions play a crucial role in determining the native state, but given their mode of action, they are not visible in solved structures (like the dog that didn't bark in the night*).
**Gregory (Scotland Yard detective): "Is there any other point to which you would wish to draw my attention?"*
Holmes: "To the curious incident of the dog in the night-time."
Gregory: "The dog did nothing in the night-time."
Holmes: "That was the curious incident."

2824-Plat
Evolution of Structure and Dynamics for a Family of Disordered Proteins
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Intrinsically Disordered Proteins (IDPs) are frequently found in vital cellular pathways including transcriptional activation and signal transduction. There is however a dearth of atomic models available for IDPs, hampering insight into how the structural ensemble is specified by the amino acid sequence. Nuclear Overhauser Effect (NOE) data was collected from the intrinsically disordered transactivation domain of a series of p53 (p53TAD) orthologues to investigate the conservation of dynamic behavior. The data suggest the presence of recurring but variable structural elements among the orthologues in the region aligning with the ubiquitin ligase MDM2 binding domain of human p53TAD, and to a lesser extent in the region correlating to the RPA binding domain of human p53TAD. The data also show significant variation in the backbone dynamics at these regions. The recurrence of these structural features across evolutionary time as suggested by the NOE data gives some credence to the idea that transient secondary structures within IDPs are constrained, though with possibly varying degrees of dynamic behavior. Future atomic modeling of the structural ensembles of these homologues should allow for a better understanding of the effect of residue similarity on the final structural ensemble in IDPs.

2825-Plat
Transient Protein-Protein Interactions in the IDP Alpha-Synuclein Detected by NMR: Implications for Protein Aggregation
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NMR paramagnetic relaxation enhancement experiments (PREs) have been applied to the intrinsically disordered protein alpha-synuclein, the primary protein in Parkinson's disease, to directly characterize transient intermolecular complexes at neutral and low pH as well as ionic strength-dependent solutions at pH 6.0. At neutral pH, we observed weak N- to C-terminal inter-chain contacts that are driven by electrostatic interactions while at low pH, C- to C-terminal inter-chain interactions are significantly stronger and driven by hydrophobic contacts. In addition to the pH-dependent transient self-associated alpha-synuclein complex, we also detected the changes of transient protein-protein interactions of alpha-synyclein in solution at varied [NaCl] (0-500 mM). By using titration-based PRE experiments, we have calculated approximate 6% and 2% transient head-to-tail complexes of alpha-synuclein in solution without and with the addition of 100 mM NaCl, respectively. The results presented here show that ^{1}H NMR paramagnetic relaxation experiments are a powerful tool for visualizing transient low-populated initial encounter complexes in intrinsically disordered proteins. Characterization of these first inter-chain interactions correlated to the aggregation kinetics will provide fundamental insight into the mechanism of amyloid formation.

Platform BA: TRP Channels

2826-Plat
Activation Mechanisms and Molecular Properties of Cyclopiazonic Acid (CPA)-Evoked TRPC Channels in Vascular Myocytes from TRPC1-/- Mice
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Phosphatidyinositol-4,5-bisphosphate (PIP2) has an obligatory role in activating heteromeric TRPC1 subunit-containing channels in vascular myocytes, which also requires protein kinase C (PKC)-mediated phosphorylation of TRPC1 proteins. The aim of the present work was to further investigate these proposed activation mechanisms in freshly isolated mesenteric artery myocytes from TRPC1-/- mice.