cytoplasm and nucleoplasm. The NPC is composed of numerous protein subunits referred to as nucleoporins, half of which are intrinsically disordered proteins (IDPs) containing numerous phenylalanine-glycine repeats. These so-called FG-nucleoporins (FG-Nups) naturally exist in an unfolded state without tertiary structure or persistent secondary structure, yet they are believed to mediate the selective gating function of the NPC. IDPs such as FG-Nups are challenging to characterize by molecular dynamics (MD) simulations because most available analysis tools were designed for protein simulations sampling relatively small conformational spaces. Our previous molecular dynamics simulations focused on exploring average static structural properties of FG-Nups such as extension, shape, and contact maps. These studies yielded significant differences in the average shapes of different FG-Nups supporting the new ''forest'' model for FG-Nup organization in the NPC. We are now extending this work to study the dynamical properties of FG-Nups, with the goal of developing statistical measures that can characterize different types and degrees of disorder from MD trajectories. We are evaluating established metrics for MD convergence and sampling efficiency (such as structural decorrelation time) as well as novel methods (such as metric scaling) to characterize the rate and extent of conformational sampling in IDPs. A significant challenge to the use of MD to study IDP dynamics is the potentially dramatic undersampling of the vast conformational space available to unstructured proteins. Therefore we are studying how to apply these analysis tools on structural ensembles derived from multiple independent simulations and accelerated MD methods. Additionally, to allow much longer MD trajectories, we are evaluating the accuracy of coarse grained force fields, such as the MARTINI model, for simulating IDPs.

1199-Pos Board B91

Synchrotron Radiation Circular Dichroism Spectroscopy of MEG14, an Intrinsically Disordered Protein

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The micro-exon genes (MEG) identified in the genome of Schistossoma mansoni, a flatworm parasite responsible for the second most widespread tropical disease, code for a class of small secreted proteins that are highly expressed in the human host stage. Due to the high variability in primary structure of the MEG proteins, the parasite is thought to recruit them to trick the host immune system; however, their specific function is still not understood. We have examined the secondary structure of MEG14, a member of this family, as well as the effects of a range of physical-chemical factors on its conformation, using synchrotron radiation circular dichroism (SRCD) spectroscopy and Oriented-SRCD. The SRCD spectrum of MEG14 in aqueous solution exhibits a strong minimum at 198 nm and a small positive band at 182 nm, corresponding a high content of disordered structure (>80%). However, disorder-to-order transitions that lead to the induction of an alpha-helix structure in MEG14 were noted by the addition of trifluoroethanol ($\geq$ 50%) with negative peak at 208 and 222 nm and a stronger positive peak at 192 with a shoulder at 175 nm. This ordering effect was also produced by increasing temperature (in a reversible process), the presence of negatively charged lipids (like POPG and SDS), and absorption on a silica surface, followed by dehydration. Taken together with consideration of its primary structure (high content of charged and proline residues, low content of aromatic and/or hydrophobic residues), these results support the classification of MEG14 as good model of an intrinsically disordered protein, and suggest the possibility of its interaction with different partners in the host to produce multifunctional roles.

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1200-Pos Board B92

Absolute Binding Affinity Calculation for MDM2 Bound to a Disordered Fragment of P53

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Accurate calculation of of protein-protein binding affinities remains a key challenge in computational biophysics and biochemistry. This challenge is even more acute when one binding partner is intrinsically disordered in its unbound state. In this study, a potential mean force (PMF) method was used to calculate the absolute binding affinity for MDM2 and the intrinsically disordered protein (IDP) fragment of p53. Angular and conformational restraints were applied to the protein during the PMF calculation, and the resulting binding affinity was corrected to account for these restraints. The calculated binding affinity closely matches experimental values and suggests that this approach could be used to determine protein-protein affinities for other IDP systems.

1201-Pos Board B93

Cyanylated Cysteine used to Examine the NTAIL/XD Bound Complex of the Nipah Virus

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Site specific vibrational probes were introduced via chemical modification of single-site cysteine mutants of the NTAIL protein of the Nipah virus. The Nipah virus NTAIL protein is intrinsically disordered but structure is induced upon binding to XD in formation of the replicative complex. Infrared spectroscopy of the introduced probe groups has been used to chart the conformational distribution of the NTAIL protein upon binding to its viral binding partner. New single cysteine mutation sites were selected to offer a thorough map of local structure in the XD-bound form, and results from these new labeling sites will be presented.

1202-Pos Board B94

Biophysical Characterization of the Multivalent Gli3/SPOP Interactions

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Contrary to traditional protein binding mechanisms, many ubiquitin ligase substrates have multiple weak degrons instead of one strong one. These proteins are largely disordered, allowing for access to each degron. The biological function of these multiple degrons is unclear. Gli3, an intrinsically disordered, zinc-finger-containing transcription factor, is an effector of Hedgehog (Hh) signaling. Gli3 degradation is mediated by the E3 cullin-RING ubiquitin ligase SPOP (speckle-type POZ protein). Ser/Thr rich SPOP-binding consensus (SBC) motifs were identified as interaction sites for SPOP. Based upon sequence analysis, Gli3 contains 13 of these motifs. Each SPOP monomer binds a single SBC motif, implying the abundance of motifs serves a function other than stoichiometric binding. This suggests several models for SPOP/Gli3 binding: (1) a dynamic complex, wherein single SPOP oligomers interact with single Gli3s in fast equilibrium; (2) a static multi-valent complex wherein each Gli3 has multiple SPOP binding partners; or (3) a high-order oligomeric complex wherein multiple Gli3 molecules interact with multiple SPOP oligomers, assembling into large complexes that can lead to phase transitions. In this study, biophysical techniques were used to probe the function of motifs in the regulation of SPOP binding. Preliminary results suggest that individual degrons have similar weak binding affinities, with no site preferentially bound. These binding sites may also play a function in the regulation of Gli3 degradation in vivo. Gli3 function as an activator or a repressor is determined by cellular Hh availability. In its active form, Gli3 enters the nucleus and, as a labile transcription activator, is rapidly degraded. The function SBC motifs play in this degradation process will be analyzed in vivo using various truncations and deletions of Gli3. Further studies will elucidate the role of multiple dispersed degrons for the temporal and spatial control of Hh signaling.

1203-Pos Board B95

Multi-Site Dependent Interaction of Hh Transcription Factor Gli3 with Cul3-SPOP Ubiquitin Ligase

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Gli3, a largely intrinsically disordered protein, is a transcriptional factor in the Hedgehog (Hh) signaling pathway. It is regulated by a Cullin-3-RING ligase that targets Gli3 for ubiquitination and degradation through its substrate recognition subunit SPOP. SPOP contains a BTB dimerization domain and a MATH domain that recognizes its substrates through Ser/Thr rich motifs, termed SPOP binding consensus (SBC) motifs. Gli3 contains 13 predicted SBC motifs. The biophysical mechanism of the interaction between dimeric SPOP and multivalent Gli3 is not well understood. To deconvolute this complex interaction we use the MATH domain of SPOP, a full-length monomeric SPOP mutant, and the SPOP dimer to characterize their interactions with a Gli3 construct spanning the N-terminal 90 residues (Gli3-90) containing three SBC motifs. To investigate stoichiometry, sites of interaction, microscopic and macroscopic affinities, we have exploited various biophysical techniques including NMR spectroscopy, fluorescence anisotropy (FA), and analytical ultracentrifugation (AUC). NMR titration experiments reveal that the predicted SBC motifs in