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mechanism by which disease-relevant alterations to tau impact its function. Together, these results draw attention to the relevance of the interaction between tau and free tubulin as playing an important role in mechanisms of tau pathology.

1377-Pos Board B107

Stathmin Exists as an Oligomer in Solution, as Evidenced by Static Light-Scattering, Native Gel Electrophoresis, and EPR Spectroscopy

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Intrinsically disordered proteins (IDPs) are an interesting class of highly dynamic, typically regulatory, proteins. They lack a native three-dimensional fold, but can often acquire a stable, ordered structure upon interaction with a binding partner. Various IDPs have been reported to exist in cells as ordered oligomers or disordered aggregates, often resulting in disease. In particular, stathmin is a regulatory IDP involved in the disassembly of cytoskeletal microtubules. As such, it is essential for proper cell function (i.e., processes coordinating the cell cycle, maintaining cell shape, etc.); improper regulation of stathmin activity has been linked to neurodegenerative diseases, mental disorders, and various cancers. It is thus important to study the solution-phase structure and conformational dynamics of stathmin, as they likely emulate the protein's behavior in cellular environments. Our preliminary static light scattering and native gel electrophoresis data suggest that stathmin may exist as an oligomer in solution, contradicting previous observations of a monomer by analytical ultracentrifugation. To investigate this further, we performed site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy on various singly-labeled stathmin mutants. The resulting EPR traces all exhibit a spectral component substantially broadened due to the dipolar interaction, implying the close proximity of two or more spin labels, likely due to dimerization or higher-order oligomerization. Upon dilution of the spin-labeled proteins with unlabeled wild-type stathmin, the spectral weight of the dipolar-broadened component was significantly reduced. The data collectively presented here support our hypothesis that stathmin exists as an oligomer in solution. These results have important implications on our understanding of the conformational dynamics of this IDP and the roles that oligomerized IDPs have in diseases.

1378-Pos Board B108

Gli3/Spop Multivalent Interactions are Concentration-Dependent

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It has been recently shown that many ubiquitin ligase substrates have multiple weak degrons instead of one strong one. These substrates are largely disordered, which allows 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 speckle-type POZ protein (SPOP). SPOP binding motifs within Gli3 were identified by peptide microarray analysis and Gli3 was found to potentially have >70 binding motifs. Each SPOP monomer binds a single SBC motif, implying the abundance of motifs serves a function other than stoichiometric binding. SPOP itself is capable of forming concentration-dependent, dynamic oligomeric complexes. In this study, biophysical and structural techniques were used to characterize the oligomeric properties of SPOP and how binding of a multivalent Gli3 substrate affects these properties. Preliminary results suggest that individual degrons have similar weak binding affinities, with no site preferentially bound. Results also show that there is a concentration threshold for interactions between Gli3 and SPOP to occur. Further studies will continue to investigate this concentration-dependence and assess how this mechanism of binding may regulate Gli3 function and correct Hh signal transduction.

1379-Pos Board B109

Solvent Effects on the Structure and Internal Dynamics of Calcitonin Gene-Related Peptide

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Calcitonin gene-related peptide (CGRP) is an intrinsically disordered, 37 residue neuropeptide that acts as a potent vasodilator. It is a member of the calcitonin peptide (Ct) family, together with amylin, calcitonin and adrenomedullin. Understanding how sequence and solvent variations affect the conformation and dynamics of these genetically and functionally related IDPs is of considerable interest. We use a nanosecond-resolved spectroscopic technique based on

tryptophan triplet quenching by cysteine to detect transient tertiary contact formation in CGRP under varying solvent and temperature conditions. Using this technique, we had previously found that electrostatic interactions modulate the degree of compaction in CGRP1. Here we explore the effect of solvent on CGRP structure and dynamics. We find that, though disordered, CGRP is very sensitive to small variations in the solution environment. Our findings can be rationalized in terms of polymer models and residual secondary structure content.

1 Sara M. Sizemore et al. 2013 Biophys. J, Supplement, Abstract.

1380-Pos Board B110

Molecular Crowding Stabilizes Both the Intrinsically Disordered Calcium-Free State and the Folded Calcium-Bound State of an RTX Protein: Implication for Toxin Secretion

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Ligand-induced disorder-to-order transition plays a key role in the biological functions of many proteins that contain intrinsically disordered domains. Here, we present data on an RTX ("Repeat in Toxin") protein, RCL, an IDP that folds upon calcium binding. RTX motifs are calcium-binding nonapeptide sequences that are found in more than 250 virulence factors secreted by Gram-negative pathogenic bacteria. Using a combination of biophysical approaches, we showed that RCL exhibits the hallmarks of intrinsically disordered proteins in the absence of calcium. Calcium binding triggers a strong reduction of the mean net charge, dehydration and compaction, folding and stabilization of secondary and tertiary structures of RCL. Moreover, RCL is an attractive model to investigate the effect of molecular crowding because it offers the opportunity to characterize the crowding effects on the same protein under two drastically distinct folding states. Macromolecular crowding affects most chemical equilibria in living cells by sterically restricting the available space. We showed that the crowding agent Ficoll70 did not affect the structural content of the apo-state and holo-state of RCL but increased the protein affinity for calcium. Besides, Ficoll70 strongly stabilizes both states of RCL, increasing their half-melting temperature (ΔT_m), without affecting enthalpy changes. The power law dependence of the ΔT_m increase on the volume fraction allowed the estimation of the Flory exponent of the thermally unfolded states. Altogether, our data suggest that, in the apo-state as found in the crowded bacterial cytosol, RTX proteins adopt extended unfolded conformations that may facilitate protein export by the secretion machinery. Subsequently, calcium gradient across bacterial cell wall and crowding also enhances the calcium-dependent folding and stability of RTX proteins once secreted in the extracellular milieu.

1381-Pos Board B111

Structural Ensembles of Intrinsically Disordered Proteins Depend Strongly on Force Field

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Intrinsically disordered proteins (IDPs) fulfill many biological roles and are important drug targets. However, they are poorly understood relative to the wealth of structural information available for globular proteins. Their structural characterization presents a formidable challenge to both theory and experiment: the structure of an IDP must be described as a structural ensemble of many interconverting conformations.

Here, we use molecular dynamics simulations to obtain structural ensembles of two IDPs: (1) a 50-residue peptide derived from Nsp1p, which is an FG-nucleoporin responsible for the selectivity of the nuclear pore complex and (2) a 20-residue RS-repeat peptide derived from serine/arginine-rich-splicing-factor 1, which is crucial in RNA splicing. Because force fields for polypeptides have been developed primarily to study folded proteins, it is not clear how accurately they can model disordered states. We therefore performed simulations using four force fields: amber99sb*-ildn, amber ff03w, CHARMM22*, and CHARMM36. We performed replica exchange (RE) simulations for a total of 150 microseconds per force field. To minimize the computational cost of these simulations, we developed an algorithm that yields a temperature ladder for which the mean first passage time between the lowest and highest temperature is minimal.

The structural ensembles we obtain for both the FG and RS peptides differ markedly between force fields with respect to hydrogen bonding, radius of gyration, and secondary structure, and are sufficiently converged to make such a comparison. Importantly, secondary structure content differs more on average between force fields than between the two peptide sequences. Thus, disordered peptides appear to be particularly sensitive to force field selection, much more so than globular proteins. A comparison to NMR data on the RS peptide is ongoing, and will shed light on which of these force fields offers the most accurate description.