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
Ashley J. Chui, Katherina C. Chua, Michael D. Bridges.
California State University, Fullerton, Fullerton, CA, USA.

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 resulting in disease is a regulatory IDP involved in the disassembly of cytосkeletal 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.

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Gli3/Spop Multivalent Interactions are Concentration-Dependent
Melissa R. Marzahn, Tanja Mittag.
Structural Biology, St. Jude Children’s Research Hospital, Memphis, TN, USA.

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 multidegrons is unclear, yet 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, implicating the abundance of motifs serves a function other than stoichiometric binding. SPOP is capable of forming concentration-dependent, dynamic oligomeric complexes. In this study, biochemical 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
Sara M. Szizemore1,2, Stephanie M. Cope1,2, Anindya Roy1, Giovanna Ghirlan1a, Sara M. Vaiana1,2.
1Center for Biological Physics, Arizona State University, Tempe, AZ, USA.
2Department of Physics, Arizona State University, Tempe, AZ, USA.
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 triplets quenching by cysteine to detect transient tertiary contact formation in CGRP under varying solvent and temperature conditions. Using this technique, we have 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.

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
Ana Cristina Sotomayor Perez, Osro Subrini, Audrey Hessel, Daniel Latand, Alexandre Chenal.
Institut Pasteur, Paris, France.

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 (∆Tm), without affecting enthalpy changes. The power law dependence of the ∆Tm 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.