317-Pos Board B117
Native State Conformational Dynamics of Disordered Protein Respond to Chemical Environment
Ucheor B. Choi, Mark E. Bowen.
Many eukaryotic proteins are predicted to be intrinsically disordered meaning that they lack stable secondary and tertiary structure. Such intrinsically disordered proteins (IDPs) are extremely flexible and exhibit large conformational fluctuations in their native state. Despite lacking the ''lock and key'' interfaces of folded proteins, IDPs are biologically functional mediating essential cellular functions like controlling access to the nucleus and synaptic vesicles. IDPs are particularly prevalent in signal transduction where many cell surface proteins contain long disordered cytosolic domains. Previously we found differences in the native state conformational dynamics using single molecule analysis. Some IDPs are truly random coils while the other showed a stochastic switching among distinct conformational states, which was indistinguishable in the ensemble measurements due to averaging of many molecules. Here we have combined single molecule and ensemble methods to characterize the solubility landscape and molecular details of polymer dynamics in different ionic compositions, phosphorylation states, and protein-protein interactions. With the ability to change structural dynamics in response to chemical environments, IDPs exhibit large structural variations which maybe linked to physiological functions.

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Molecular Recognition Features Facilitate the Binding Diversity of Hub Proteins
Wei-Lun Hsu.
Molecular recognition features (MoRFs) are short, unstructured regions that perform molecular binding and recognition functions via disorder-to-order transitions. These short disordered peptides have various names in the literature, such as ELMs (eukaryotic linear motifs), SLiMs (short linear motifs) and ANCHORS (predicted disordered binding sites based on energy calculation). The MoRF mechanisms are hypothesized to involve in the binding diversity of hub proteins in two ways: one-to-many signaling in disordered hubs (e.g. p53) and many-to-one signaling in ordered hubs (e.g. 14-3-3). The former hub uses the same disordered region to form different secondary structures to bind four structurally different partners. The latter hub binds to five distinct disordered regions via one structured binding groove. Our work explored hundreds of hub protein examples by analyzing the complexes deposited in Protein Data Bank that have same binding MoRFs or similar binding grooves. Further computational experiments and analyses provided us more detailed and specific explanations regarding how disordered regions facilitate the binding diversity in different complex structures. Exploring more examples absolutely gave us much clearer picture to uncover the conformational changes that occur accompanying with binding, and showing that, in general, flexibility allows both subtle and complex structural variation thereby enabling the same sequence to morph into differently shaped binding sites and different sequences to fit into the same binding site. These results not only verify the two MoRF mechanisms we observed in the single examples of one-to-many and many-to-one signaling previously, but also generalize the specific examples to many hundreds of other well-characterized protein-protein interactions.

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Elasticity of Intrinsically Disordered Nebulin Modules
Jeffrey G. Forbes, Richard J. Wittebort, Alexander Grishaev, Wanxia L. Tsai, Kuan Wang.
The elasticity of native full length nebulin, demonstrated recently via atomic force microscopy with site-specific antibody pairs as force handles (Langmuir, 2009, 25, 7496), suggests that in the thin filaments, nebulin is stretched to cope with the actin length and imposes significant force and influences the functions of the underlying actins. This pre-stressed mechanical state of thin filaments may have important implications for the role of nebulin as a length ruler and as a regulator of actomyosin interaction. The structural basis of nebulin elasticity remains open. We report here the structural characterization of modules from the super-repeat and single repeat regions by a combination of circular dichroism (CD), NMR, SAXS, AFM, structural predictions and steered molecular dynamics simulations. In aqueous solutions of common buffers, these modules are intrinsically disordered, but are poised to form alpha-helices, especially in the presence of trifluoroethanol. SAXS analysis of a four-module construct indicates an elongated structure with a radius of gyration of 3.6 nm and, as modeled with DAMMIN, shows a contour length of ~15 nm. Interestingly, this extended structure is also evident in a small population of the structural models as predicted by ROSETTA++. AFM images of the modules on an inert surface are predominantly compact with an average height of ~ 2.5 nm, consistent with the bulk of the ROSETTA predictions. These structural ensembles of compact and extended structures are significantly shorter than what it would take for nebulin modules to wrap around the perimeter of actin filaments (~6 nm per module). We propose that nebulin modules' disorder-order transition of alpha helices, contributes to its elasticity and how nebulin juxtapositions itself onto the actin to form a pre-stressed thin filaments in the muscle sarcomere.

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Alanine Scan of a Small Molecule Interaction Site on the Disordered c-Myc Oncoprotein
Lisette M. Fred, Bethany L. Zablotsky, Kaitlyn P. Gerhart, Steven J. Metallo.
The transcription factor c-MYC is an intrinsically disordered oncoprotein that undergoes coupled folding and binding to its obligate dimerization partner MAX. Upon dimerization c-MYC and MAX form a basic helix-loop-helix leucine zipper conformation. Many forms of cancer have been associated with the overexpression of c-MYC. Previously we have identified three independent small molecule binding sites on c-MYC that stabilize the disordered, monomeric form and inhibit dimerization with MAX. One of these small molecule inhibitors, 10074-G5, binds specifically to a short amino acid sequence in helix 1 of the monomeric c-MYC. Each amino acid in this binding region was individually changed to an alanine residue in order to investigate the binding contributions for each amino acid of the site. Substituting the amino acids of the binding site for alanine may also alter the conformation of the inhibitor binding site, innately changing the c-MYC affinity for 10074-G5. Using this method to analyze the direct and indirect amino acid contributions to 10074-G5 binding will not only provide further insight into defining the functional epitope of the 10074-G5 binding site of c-MYC, but also provide insight to the conformational requirements to maintain or enhance inhibitor affinity.

321-Pos Board B121
Tri-Aspartic Acid Peptides in Water: A Suitable Model System for Determining the Structural Propensities of DxD Motifs in Unfolded Proteins
Laura Duitch, Siobhan Toal, Daniel Verbaro, Andrew Hagarman, Reinhard Schweitzer-Stenner.
In the context of our ongoing investigations of the conformational propensities of amino acid residues in unfolded peptides we recently focused on the peculiar properties of aspartic acid (D). A detailed spectroscopic study on the tripeptide GDG has shown that D has an unusual preference for turn like structures. This is interesting since D has been shown to be involved in so called asx -turns which are stabilized by hydrogen bonding between the polar residue of e.g. D and the adjacent amide proton. D is also an essential ingredient of DxD motifs which are involved in the enzymatic activity and the Golgi glycosyltransferase GM2. To shed some light on how D-residues behave in D3-motifs we re-investigated the tripeptide D3 with IR, polarized Raman, vibrational circular dichroism, electronic circular dirchroism and NMR spectroscopy. The data are currently being analyzed. First results indicate that D3 has more PPII content than GDG at room temperature and that its actual structure depends on the protonation state of its residues. At high temperature the ECD spectra is indicative of a predominant sampling of right-handed helix or (type III β) turn like conformations, which is rather unexpected. Our results indicate that aspartic acid combines a high degree of plasticity with a preference for turn structures which might explain its role in turn forming segments in proteins and the functional properties of DxD motifs.

322-Pos Board B122
Unexpected Features of Polymer Partitioning from Semi-Dilute Mixtures Into Protein Voids
Philip Gurnev, Rudolf Podgornik, Joel A. Cohen, Sergey M. Bezrukov, **Adrian Parsegian**.
Molecular crowding creates new modes of polymer transport. Polymers at high concentration are able to penetrate ionic channels much narrower than the nominal radii of polymer gyration. Partitioning between bathing solution and molecular cavities reveals additional interaction and interference. Here we look at mixtures of two polymers, polyethylene glycols (PEGs) of MW 200 and 3400, that are presented with the possibility of going into an alpha-hemolysin pore. The smaller PEGs partition freely, with the same concentration in the channel (measured by conductance) as outside (gauged by conductivity). The larger polymers stay outside, up to a weight concentration of 15%; then episodically (showing large conductance fluctuations) they start to enter until, at 30 wt% concentration, they too approach a concentration that is equal inside and outside the channel. In a mixed PEG200/3400 solution, PEG 200 partitions disproportionately into the channel, with the extra push of the almost-completely excluded PEG3400. We observe partitioning for a range of polymer ratios and find a remarkable conundrum. Pure polymers, big or small, partition completely into the pore at 30 wt%. Surprisingly, chopping a minor fraction of the 3400 MW PEGs into 200 MW pieces actually reduces the total partitioning of polymers, a reduction that persists until almost all the PEG is 200 MW.