Intrinsically Disordered Proteins II

3392-Pos
Intrinsic Disorder, Scaffolds, and Stochastic Machines
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Scaffold proteins bind additional proteins that then carry out multi-step pathways. How do such machines work? Here a new hypothesis is proposed for the complex consisting of axin, two kinases - GSK3β and CK1α - and β-catenin. The pathway involves four discrete phosphorylations of β-catenin by the kinases. Like many other scaffold proteins, axin is mostly unstructured [1]. With a length of about 800 residues, axin forms two small domains of less than 100 residues each, and uses only a small number of residues, about 20 per interaction, to bind to GSK3β and β-catenin [1], and presumably also to bind to CK1α. Thus, even with the two domains and 3 partners, axin remains mostly unfolded. The hypothesis is that the unstructured axin molecule holds the three globular proteins in very high local concentrations, like three globules on a rope, and that, by random motions, first CK1α and then GSK3β phosphorylate the disordered tail of β-catenin successively four times. The ''conformational changes'' of axin that lead to acceleration of phosphorylation are neither specific nor coordinated, but rather are entirely stochastic, with stereochemical fit between the enzymes and their targets leading to the correct ordering of the four phosphorylation steps. In this hypothesis, the scaffold protein acts simply as a flexible tether that leads to acceleration of the multiple steps in the pathway by raising the local concentrations of the key components and by allowing the various components the freedom to collide in various orientations until productive collisions result. Thus, the steps of the pathway are carried out by a stochastic machine. This may be a general mechanism for scaffold-based molecular machines.
[1] Cortese, MS, Uversky, VN, and Dunker, AK. Intrinsic disorder in scaffold proteins: getting more from less. Prog. Biophys. Mol. Biol. 98: 85-106 (2008)

3393-Pos
Disorderness Profile of Scaffolding Proteins as a Predictor of Supramolecular Architecture: Titin and Nebulin Profiles Correlate with the Sarcomere Proportion
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Intrinsically disordered proteins often are involved in coupled folding and binding or as flexible linkers between globular domains, and take part in many important cellular functions. Predicted disorderness profiles of individual proteins often correlate well with their structural features. The most extended intrinsically disordered regions are found in the giant proteins titin (3-4MDa) and nebulin (~800kDa) in striated muscle that have long been believed to play a scaffolding or templating role in organizing the sarcomere structure. We are exploring whether disorderness profiles of these giant proteins contain instructions for the architecture of the sarcomere. The disorder profiles of titin and nebulin are complex; however, segments that are clearly disordered or ordered are readily identified. We applied a recently developed adaptive data analysis method (Hilbert-Huang Transform) to identify hidden periodicities in the disorder probability profiles of titin and nebulin. The method extracts intrinsic mode functions (IMFs), each with a small range of periodicity. This method works on the types of data where Fourier analysis fails and is ideally suited for the disorderness profiles and clearly shows trends that are hidden by other signals. Analysis of titin and nebulin isoforms have allowed us to identify IMFs and other HHT parameters that can be used to gauge the landmarks on the profiles that correlate with the architectural features of the sarcomere, such as the length of thick- and thin-filaments, the degree of overlap and the thickness of the Z-bands. We propose that the intrinsic disorder of these giant proteins may play a role in guiding the assembly of the muscle sarcomere. It is conceivable that profiles for other scaffolding proteins in general may contain the instructions for the organization of their supramolecular complexes.

3394-Pos
Dynamics of a Skeletal Troponin C - Troponin I Chimera Probed by Comparison of Experimental and Simulated NMR Relaxation Parameters
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The activation of skeletal and cardiac muscle is triggered by the release of calcium from the sarcoplasmic reticulum. The calcium sensor is the troponin complex that is formed by three subunits: the calcium-binding protein troponin C (TnC), the inhibitory protein troponin I (TnI) and the tropomyosin-associated protein troponin T (TnT). When calcium binds to TnC, the resulting conformational change allows TnC to bind TnI, leading to the removal of the C-terminal region of TnI from actin. Consequential movement of the tropomyosin allows the binding of the myosin head to actin resulting in a power stroke. Regions of these proteins are highly flexible and the importance of these intrinsically disordered sections has been recently recognized and rationalized (Hoffman et al. J. Mol. Biol. 2006 361:625-633).
Structural studies of the muscle system have been very successful in determining the structural organization of most of the molecular components involved in force generation at the atomic level. Although mainly α-helical, the structure and dynamics of TnI remains controversial, particularly in its C-terminal region. Different structures have been presented for this region: a single α-helix observed by x-ray crystallography, a ''mobile domain'' containing a small β-sheet derived from NMR restraints, and a mainly unstructured region according to NMR relaxation data. To investigate this, we have constructed a skeletal TnC-TnI chimera that contains the N-domain of TnC (1-91), a short linker (GGAGG), and the C-terminal region of TnI (98-182). Our objective is to determine which of the three proposed structures best fit the experimental 15N relaxation data for this chimera. The comparison between experimental and NMR relaxation parameters calculated from molecular dynamic simulations will be presented to assess the validity of the three models.

3395-Pos
Competition and Complex Formation Between P53, Mdm2 and the P300 Zinc Finger CH3
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The transcription factor p53 plays a crucial role in protecting cells from cancerous transformation. Its activity is primarily modulated through its N terminal interactions with the negative regulator Mdm2 and with the coactivator p300; the intrinsically disordered N terminus of p53 is the essential link between target gene binding by p53 and its subsequent expression. Therefore, a comprehensive, atomic-level understanding of these interactions is crucial for us to fully understand p53 activation. Previous work in our laboratory has shown that phosphorylation of the p53 N terminus is responsible for promoting binding to p300 (and subsequent p53 survival) at the expense of Mdm2-mediated degradation; however other laboratories have postulated a system of ternary complex formation whereby Mdm2 and CH3 can bind simultaneously to a single p53 N terminus. In order to resolve this issue, we have conducted an in-depth biophysical analysis of the p53 N terminus interactions with the zinc finger CH3 domain of p300 and the N terminus of Mdm2. We present a detailed analysis of the competition between the CH3 domain of p300 and the Mdm2 N terminus for the p53 N terminus. We have probed the thermodynamics of the system using isothermal titration calorimetry and have used mass spectrometry and multiangle light scattering to investigate the formation of complexes under a range of conditions.

3396-Pos
Structural Differences Between Apolipoprotein E3 and E4 as Measured by ^{19}F- NMR
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The apolipoprotein E family contains three major isoforms (ApoE4, E3 and E2) that are directly involved with lipoprotein metabolism and cholesterol transport. These isoforms differ in only two positions with apoE4 containing arginine residues at positions 112 and 158 while apoE3 contains a single cysteine (Cys^{112}, Arg^{158}), and apoE2 contains two cysteines (Cys^{112}, Cys^{158}). Yet only apoE4 is recognized as a risk factor for Alzheimer's disease. Here we use ^{19}F-NMR to compare structural differences between apoE4 and apoE3 and the effect of the C-terminal domain on the N-terminal domain. Both proteins contain 7 tryptophan residues and we have incorporated 5-^{19}F-tryptophan into these proteins and examined the 1D ^{19}F-NMR spectrum. NMR resonances of the wild-type proteins are broad and overlapping but show that at least 4 tryptophan residues appear to be solvent exposed while three resonances, arising from the N-terminal region of the protein, are buried. Similar results were obtained with apoE containing 4 mutations in the C-terminal region that gives rise to a monomeric form either of apoE3 under native conditions [Zhang et al. Biochemistry (2007) 46 10722-10732] or apoE4 in the presence of 1 M urea. For either wild-type or mutant proteins the differences in tryptophan resonances assigned to residues in the N-terminal region of the protein suggest structural differences between apoE3 and apoE4 as a consequence of the Arg158Cys mutation and as a consequence of the presence of the C-terminal domain. We postulate

that differences in structure in the N-terminal domain may account for differences in stability of the two proteins.

3397-Pos

Light Chain-Mediated Self-Association of Intrinsically Disordered Dynein Intermediate Chain

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Cytoplasmic dynein is a microtubule-associated protein with functions in cell division, positioning of organelles and the transport of cellular molecules. The dynein complex is composed of six subunits; but how these subunits assemble to form a functional complex is not entirely clear. In an on-going effort to understand complex assembly in cytoplasmic dynein, we have initiated structural studies of three of the subunits; IC 74, the intermediate chain subunit and its light chain binding partners LC8 and Tctex. These three subunits form a tight sub-complex at the base of the dynein particle where they are presumed to function as cargo adaptors or regulate the assembly of the complex.

We have previously reported that binding of LC8 to the intrinsically disordered N-terminal domain of IC 74 leads to helix formation in a region downstream of the binding site. To better assess these structural changes several cysteine mutations that allowed monitoring of specific segments of the helix forming region were introduced into an IC 74 construct (IC_{TLCC}). Fluorescence experiments on fluorophore-labeled IC_{TLCC} show that binding of the light chains induce self-quenching of the fluorophore. We attribute this self-quenching to close proximity of the IC_{TLCC} chains likely due to a modest IC-IC self-association. This modest IC-IC self-association is not observed in the absence of light chain binding. Thermodynamics of the IC 74-light chain interactions indicate that while binding of LC8 to IC_{TLCC} is moderately weak (10 μM), having a pre-formed IC_{TLCC} cysteine cross-linked dimer or Tctex pre-bound to the IC_{TLCC} construct enhances the binding affinity (0.1-0.2 μM). Taken together, these results are consistent with a model where light chain binding coupled to IC-IC self-association could be important for stabilizing the dynein complex.

3398-Pos

Aquifex Aeolicus FlgM Protein Does Not Exhibit the Disordered Character of the Salmonella Typhimurium FlgM Protein

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Studies on the nature and function of Intrinsically Disordered Proteins (IDP) over the past ten years have demonstrated the importance of IDPs in normal cellular function. The flexibility of IDPs allows one IDP to assume multiple conformations or form different protein-protein complexes, allowing a single protein to exhibit multiple functions. While many predicted IDPs have been characterized on an individual basis, the conservation of disorder between homologous proteins from different organisms has not been carefully studied. We now demonstrate that the FlgM protein from the thermophile Aquifex aeolicus exhibits significantly less disorder then the previously characterized FlgM protein from Salmonella typhimurium. FlgM is an inhibitor of the RNA transcription factor σ28, which is involved in regulation of flagella synthesis gene expression. Previous work has shown that the S. typhimurium FlgM protein is an intrinsically disordered protein, though the C-terminus becomes ordered when bound to σ28 or under crowded solution conditions. In this work, we demonstrate that, even under dilute solution conditions, that the A. aeolicus FlgM protein exhibits alpha-helical character. Furthermore, we use the fluorescent probe FlAsH to show that the H2 helix is ordered, even in the unbound state, in contrast to the S. typhimurium FlgM protein, and the H1 and H2 helices appear to be associated in the absence of the σ28 protein. Taken together, our data demonstrates that the A. aeolicus FlgM protein, while flexible, does not exhibit the intrinsically disordered nature exhibited by the S. typhimurium FlgM protein.

3399-Pos

Analyzing the Self-Organizing Mechanism of Lysozyme Amyloid Fiber Formation

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The formation of amyloid fibers involves a number of different intermediates. By using separation techniques and analysis methods such as dielectric spectroscopy, AFM, and TEM, the aggregation steps of fiber formation were analyzed. An Agilent 4294A impedance analyzer and an Agilent 16452A liquid test fixture over a frequency range of 40Hz to 30MHz was used for dielectric spectroscopy. We approach amyloid fiber formation using the newly introduced colloidal model [1]. This model suggests that proteins aggregate into uniformly sized nano-spheres, driven by surface energy minimization. The uniform spheres then behave like a mono-dispersed colloidal suspension. Once the spheres have reached their critical diameter it is observed from microscopy that the colloidal growth stops. At this point the attractive forces that favor agglomeration are balanced by the barrier potential forces that retard agglomeration. The fully developed nucleation units then assemble in a linear fashion before finally evolving into mature amyloid fibers. The model postulates that the linear assembly arises from dipole-dipole interaction between nano-spheres. We analyze this assembly process in vitro using lysozyme from chicken egg whites in an acidic environment. In vivo, lysozyme has a propensity to form amyloid fibers in systemic amyloidosis diseases. Lysozyme amyloid fibers are synthesized in vitro and separated into samples according to particle size. Our separation techniques yielded three samples: 1. a solution with a high concentration of monomeric lysozyme and small oligomers, 2. a solution composed of colloidal spheres and short fibers, and 3. a solution with a high concentration of mature amyloid fibers. The existence of these species in the three samples was confirmed with AFM, TEM, and Thioflavin-T binding assays. Results of dielectric analysis indicate intermediate sized aggregates have a higher dipole moment than small aggregates.

[1] S. Xu, Amyloid, 14, 119 (2007).

3400-Pos

Early Oligomer Formation of Alpha-Synuclein As Revealed by Fluorescence Correlation Spectroscopy

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Here we study the formation of early oligomers of α-synuclein by applying Fluorescence Correlation Spectroscopy (FCS). The idea is to use trace amounts (nM) of labeled protein in the presence of a large excess of unlabeled protein and follow the aggregation process by measuring the reduction in time of the diffusion coefficient of the fluorescent species. Synuclein with an engineered cysteine (A140C) was labeled with Alexa488 and was used as a fluorescent probe in trace amounts (3-4 nM) in the presence of 100 μM unlabeled α-synuclein. The combination of short sampling times and repeated measurements produce a size distribution of the oligomers. Initially, a sharp peak is obtained (diffusion coefficient 114 ± 15 μm^2/sec) corresponding to monomers. Subsequently a sharp transient population appears, followed by the gradual formation of broader sized distributions of higher oligomers. The process can be studied in time by following the reduction of the apparent monomer concentration. (Big aggregates are moving too slow to contribute to the fluctuations). The kinetics of this process can also be fitted with the Finke-Watzky equation for a two state- two step mechanism (Morris et al., Biochemistry 2008, 47:2413-27), but the rate constants obtained from this process are different from the rate constants for turbidity formation, indicating the need for an intermediate state. The formation of the transient intermediate and the early oligomers is accompanied by a conformational change, as visualised using FRET between the donor labeled N-terminus and the acceptor labeled cysteine at position A140C.

3401-Pos

Structural and Functional Insights Into Lipid Binding by Oligomeric Alpha-Synuclein

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Oligomeric alpha-synuclein is considered to be the potential toxic species responsible for the onset and progression of Parkinson's disease, possibly through the disruption of lipid membranes. Although there is evidence that oligomers contain considerable amounts of secondary structure, more detailed information on the structural characteristics and how these mediate oligomer-lipid binding are critically lacking. We have used tryptophan fluorescence spectroscopy to gain insight into the structural features of oligomeric alpha-synuclein and the structural basis for oligomer-lipid interactions. Several single tryptophan mutants of alpha-synuclein were used to gain site-specific information about the microenvironment of monomeric, oligomeric and lipid bound oligomeric alpha-synuclein. Acrylamide quenching and spectral analyses indicate that the tryptophan residues are considerably more solvent protected in the oligomeric form compared to the monomeric protein. In the oligomers, the negatively charged C-terminus was the most solvent exposed part of the protein. Upon lipid binding a blue shift in fluorescence is observed for alpha-synuclein mutants where the tryptophan is located within the N-terminal region. These results suggest that as in the case of monomeric alpha-synuclein, the N-terminus is critical in determining oligomer-lipid binding. We have further systematically studied the influence of the physical membrane properties and solution conditions on lipid bilayer disruption by oligomeric alpha-synuclein using a dye release assay, and have quantitatively studied oligomer lipid binding by confocal fluorescence microscopy and fluorescence correlation