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)

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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.

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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.

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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.

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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