their respective receptors in different organs and carry out different functions. Amylin is involved in regulating glucose metabolism and is implicated in type II diabetes, while CGRP is a vasodilator involved in transmitting pain signals in the nervous system, and triggers migraine attacks. Amylin and CGRP share 47% sequence homology and are able to bind to each other's receptors and activate cell response. Such cross-reactivity is attributed to their possible structural similarity. Solution state NMR experiments show that both peptides are disordered and locally sample transient helical states close to the N-terminus. While such short-range structural properties have been compared, it is not clear whether the long-range properties are affected or not. Here we combine results from experiments, probing both long- and short-range properties of the two peptides, with results from replica exchange molecular dynamics (REMD) simulations. To measure a long-range property directly comparable to simulations, we use a nanosecond laser-pump spectroscopy technique based on tryptophan triplet quenching. This allows probing both the end-to-end distance and the rate of end-to-end contact formation in IDPs, without using prosthetic dyes. Because of the short length of our peptides and the high aggregation propensity of amylin, this information cannot be obtained using other techniques such as FRET. Our data show that both the secondary structure content and the end-to-end distance of the two peptides differ significantly, and that such differences are affected by electrostatic interactions. Both our experiments and REMD simulations indicate that long-range interactions (i.e. interactions between residues that are far away in the sequence), play a significant role in determining the peptide structural ensemble in solution.

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Primary Sequence Controls the Specificity and Affinity of a Small Molecule Binding to the Intrinsically Disordered Protein c-Myc

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Intrinsically disordered proteins (IDPs) are characterized by high flexibility and low hydrophobic to charged residue ratio. The transcription factor c-Myc is an IDP deregulated in many forms of cancer. The protein undergoes coupled folding and binding with its obligate dimerization partner Max, which is also a disordered monomer, to form a basic helix-loop-helix leucine zipper (bHLHZip). A small molecule, 10058-F4, binds specifically within an 11 amino acid region of the bHLHZip of c-Myc, stabilizing the disordered monomer. The affinity determining residues of the 10058-F4 binding site on c-Myc were distinguished by mutating individual residues to alanine and subsequently measuring binding of 10058-F4. Mutation of both hydrophobic and certain hydrophilic residues attenuated binding of the small molecule to c-Myc. The affinity determining residues may affect binding through direct, energetically favorable contact with the small molecule or via a conformational influence on the IDP which favors binding. Within the proteome (SLiMSearch3), only two proteins are identified with five affinity determining residues. Six affinity determining residues are enough to specify c-Myc. A minimal set of these key residues were introduced into Max, which does not normally interact with 10058-F4. The novel protein, ModMax, binds 10058-F4. Although the alanine scan distinguished the necessary amino acids for binding, conservative mutations of some affinity determining residues demonstrated that 10058-F4 affinity is tunable. Upon substitution of Y to W, the affinity was improved by an order of magnitude. On the other hand, the affinity decreased five-fold upon substitution of E to N. Primary sequence alone, without extended secondary and tertiary structure, is sufficient to confer both specificity and affinity of a small molecule-IDP interaction.

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Interaction of the Intrinsically Disordered c-Myc Oncoprotein with Racemic and Enantiopure Small Molecules

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The prevalence of intrinsically disordered proteins (IDPs) in cell signaling and disease makes them significant targets. Despite the absence of defined tertiary structure, small molecules can bind IDPs at sites determined by a short, linear segment of the protein's primary sequence. The oncoprotein c-Myc, a transcription factor that must undergo coupled folding and binding to its obligate partner Max in order to interact with DNA, is an ideal system for understanding specificity in small-molecule binding to IDPs. Three small molecule interaction sites exist in the bHLHZip region of c-Myc, the segment necessary for coupled folding and binding to Max. The chiral small molecule 10074-A4 interacts with one of these sites (Myc372-389). The presence of Myc372-389 induces small molecule circular dichroism of racemic 10074-A4, indicating an enantiospecific interaction. We have synthesized the pure R and S enantiomers of 10074-A4 (as well as pure enantiomers of derivatives) and found that at 10 uM and above the compound can undergo a transition upon addition to water from an aggregate, to a dispersed molecule, to an assembled chiral complex with a strong CD signature. SPR measurements indicate interaction between Myc and 10074-A4. These data suggest multiple possible binding modes. We also report the hydrodynamic radius of the bHLHZip region of c-Myc, as determined by fluorescence correlation spectroscopy and dynamic light scattering, under different conditions including in the presence of various small molecules and c-Myc's obligate partner Max.

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The Intrinsically Disordered C-Terminal Tails of E. coli Single-Stranded DNA Binding Protein Regulate Cooperative Binding to Single-Stranded DNA

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E. coli single strand DNA binding protein (SSB) is one of the key proteins in DNA replication, recombination and repair. SSB functions as a homotetramer and binds ssDNA in different modes using either all four subunits ($(SSB)_{65}$ mode) or two subunits ($(SSB)_{35}$ mode), which are regulated by salt concentration and SSB binding density. These binding modes display very different ssDNA binding properties with $(SSB)_{35}$ mode showing highly cooperative binding. Each SSB subunit (177 amino acids) consists of two domains: an N-terminal DNA binding core containing an oligonucleotide/oligosaccharide binding (OB) fold (residues 1-112) and an intrinsically disordered (ID) C-terminal tail (65 residues). While the conserved last nine amino acids of the C-terminal tail (''the tip'') provide the site for interaction with more than a dozen metabolic proteins the role of the ID linker (56 amino acids) remains unclear. Here we show that the amino acid composition and length of the IDL affects the ssDNA binding mode preferences of SSB protein. Surprisingly the number of IDLs and the lengths of individual IDLs together with the acidic tip contribute to highly cooperative binding in the $(SSB)_{35}$ binding mode. Atomistic simulations suggest that cooperative binding correlates with preference of IDLs for globular conformations (supported by NIH grant GM030498 (TML) and NSF MCB 1121867 (RVP)).

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Assessing Binding Perturbation due to Artificial Vibrational Probe Groups in the Nucleoprotein-Phosphoprotein Complex of the Nipah Virus

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The binding interaction between the intrinsically disordered nucleoprotein tail and the phosphoprotein of the Nipah Virus (NiV) involves both disorder-to-order transition and fuzzy binding. To examine the dynamic structure and the conformational distribution of this interaction, a site-specific thiocyanate (SCN) vibrational probe was incorporated at many sites on the binding region of the NiV NTAIL. Since this binding is likely driven by hydrophobic forces, replacing a non-polar amino acid side chain with a polar probe could perturb binding. Isothermal titration calorimetry (ITC) experiments were designed to determine the extent of disruption to binding thermodynamics. The ITC results were then used to inform the interpretation of the vibrational spectroscopy data and measure the importance of single amino acids in maintaining this ''fuzzy'' binding interface.

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Claws, Disorder, and Conformational Dynamics of the C Terminal Region of Human Desmoplakin

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Cellular adhesion is governed by desmosomes, which are large inter-cellular junctions that act by tethering the intermediate filaments of separate cells. Intermediate filaments bind to a protein known as desmoplakin (DP), which in turn is linked to a membrane-bound cadherin complex. The serine-rich C-terminal region of DP was recently shown to modulate its binding to intermediate filaments. Several phosphorylation sites on the C-terminal region have been identified via mass spectrometry, and have been shown to regulate the binding strength. To elucidate the molecular mechanism of this coupling, and the role of specific post-translational modifications (PTM), we are using molecular dynamics simulations to examine the structural behavior of several forms of desmoplakin (wild-type, mutants, with and without PTMs). Our results indicate