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immune system activation. Alterations in the regulation of CaN can lead to disorders such as Down syndrome-related mental retardation and cardiac hypertrophy. CaN is also the target for the immunosuppressant drugs FK506 and cyclosporin A. The regulation of CaN function is not well understood at the molecular level. CaN is inactive until bound by calmodulin (CaM). CaM binds at a site towards the N-terminus of a 95 residue regulatory domain in CaN. This regulatory domain is believed to be disordered. The binding of CaM to CaN causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. We hypothesize that the CaN regulatory domain undergoes a folding transition upon CaM binding, and that this folding provides the driving force for pulling the autoinhibitory domain from the active site. We have made a fragment of CaN that consists of the regulatory domain, autoinhibitory domain and a short C-terminal domain. We will present data from CD spectroscopy, fluorescence, NMR and analytical ultracentrifugation experiments that indicate this fragment is largely disordered in the absence of CaM, and gains structure when CaM binds.

1135-Plat
Bioinformatic Analysis of the Role of Intrinsic Disorder in Multiple Specificity
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Several lines of evidence suggest that intrinsically disordered proteins (IDPs) are a common mechanism used by nature to mediate protein-protein interactions. IDPs lack a stable three-dimensional structure under physiological conditions and many such proteins have been characterized by several biophysical methods. Additionally, IDPs are estimated to be abundant within various proteomes, particularly eukaryotes, and carry out a variety of molecular functions without the prerequisite of a specific, stable structure. It is thought that IDPs can facilitate protein interactions through an ability to mediate binding diversity, where one of the proposed mechanisms for this is multiple specificity - i.e. recognition of multiple molecular partners through use of the same binding residues - through contextual folding of IDPs.
In previous work, two contrasting examples of proteins with multiple binding specificity were examined, 14-3-3ζ and p53, which exemplify the potential of intrinsic disorder for mediating protein interactions. 14-3-3ζ has a structured domain with a single binging pocket that is responsible for the binding of various protein partners through interaction with sequence divergent intrinsically disordered segments in these partners. In contrast, the disordered termini of p53 contain discrete regions that are each involved in many interactions with different protein partners, where these interactions carry out and regulate p53 function. The common theme in both of these examples is structural variability in the bound state that is enabled by intrinsic disorder in one of the partners in the unbound state.
In current work, the previous analysis is expanded to many other examples of proteins that interact with multiple partners using a common binding site. These data support the conjecture that intrinsic disorder enables binding to multiple partners and provides detailed information about induced fit in structured regions.

1136-Plat
High-throughput Characterization of Intrinsically Disordered Proteins from the Protein Structure Initiative
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The identification of intrinsically disordered proteins (IDPs) among the targets that fail to form satisfactory crystal structures in the Protein Structure Initiative represent a key to reducing the costs and time for determining three-dimensional structures of proteins. To help in this endeavor, several Protein Structure Initiative Centers were asked to send samples of both crystallizable proteins and proteins that failed to crystallize. Initially, the abundance of intrinsic disorder in these proteins was evaluated via computational analysis using Predictors of Natural Disordered Regions (PONDR®) and the potential cleavage sites and corresponding fragments were determined. Then, the target proteins were analyzed for intrinsic disorder by their resistance to limited proteolysis. The rates of tryptic digestion of sample target proteins were compared to those of myoglobin, apomyoglobin and α-casein as standards of ordered, partially disordered and completely disordered proteins, respectively. Results from these digestion experiments generally correlated with the results of disorder predictions. At the next stage, the protein samples were subjected to both far-UV and near-UV circular dichroism (CD) analysis. For most of the samples, a good agreement between CD data, predictions of disorder and the rates of limited tryptic digestion was established. Most samples corresponded to proteins that were predicted to be ordered had slower digestion rates and showed a good amount of ordered structure as determined by near- and far-UV CD analysis. On the contrary, predicted to be disordered proteins were digested fast and possessed spectral features characteristic of IDPs. Further experimentation is being performed on a smaller subset of these samples in order to obtain more detailed information about the ordered/disordered nature of the proteins.

1137-Plat
How Does Charge Content Modulate Conformational Equilibria of Intrinsically Disordered Proteins? An Illustration Using Protamines
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Intrinsically disordered proteins (IDPs) adopt heterogeneous ensembles of conformations at equilibrium under physiological conditions. Just as the structure of a folded protein determines its function, the conformational ensemble of an IDP governs its interactions with binding partners. We seek quantitative descriptions of conformational equilibria anchored in polymer physics concepts that capture the richness of IDP phase diagrams. Recent studies by our lab showed that archetypal polar homopolymer IDPs favor collapsed ensembles in water despite the absence of hydrophobes, a counterintuitive result that even held for polypeptide backbones alone. We now turn our attention to highly charged peptides, which constitute a different archetype of IDP. We simulated a variety of protamines - a class of arginine-rich IDPs involved in the condensation of nuclear chromatin during spermatogenesis - in aqueous 125 mM salt solutions in order to elucidate the influence of charge content on conformational equilibria. The simulations were performed with ABSINTH, a Monte Carlo engine that employs our recently-developed implicit solvation model. We find that protamines with high charge asymmetry are similar in their adoption of extended bent-rod conformations, a result in agreement with theoretical predictions. Sequences with identical charge asymmetry but different charge composition exhibited similar characteristics in terms of overall size measures such as radius of gyration. However, local properties such as alpha helix propensity remained strongly dependent on the particular sequence. These findings point towards a possible engineering principle for IDP sequence design: general size requirements set the charge asymmetry, while local conformational specifications govern the particular sequence. This principle is consistent with the evolutionary pattern of protamines: while sequences exhibit hypervariability across species, arginine content is highly conserved.
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1138-Plat
Characterization of the Unfolded State Under Native Conditions: A Missing Piece of the Protein Folding Puzzle
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The nature of the denatured state ensemble is controversial owing in large part to the difficulty of characterizing the structure and energetics of denatured state interactions. Denatured states can be populated under a variety of extreme conditions but the state which is most relevant for protein folding and engineering is the denatured state ensemble which is populated in the absence of denaturant under native conditions. Unfortunately this state is usually experimentally inaccessible. We reported detailed characterized of the denatured state populated under native conditions for two α-β proteins, the N-terminal domain of the ribosomal protein L9 (NTL9) and the C-terminal domain of the same protein (CTL9) , as well as for a rapid folding all helical structure the villin headpiece helical subdomain, HP-36. Conditions have been found where the native and denatured states of CTL9 are both populated in the absence of denaturant and 1H, 15N and 13C NMR was used to define the conformation propensities of the denatured state. For NTL9 the thermodynamic linkage between proton binding and protein stability was used to characterize denatured state electrostatic interactions. Peptide models were exploited to characterize the denatured state of HP-36. In all three cases, the denatured states contains significant structure. The impact of this preformed structure on the kinetics and mechanism of protein folding is discussed.

1139-Plat
Distribution of Conformations Sampled by the Central Amino Acid Residue in GXG Peptides Inferred from Amide 1′ Band Profiles and NMR Scalar Coupling Constants
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