Intrinsically Disordered Proteins I

1332-Pos
Effects of pH on Conformational Equilibria of Intrinsically Disordered Proteins
Albert H. Mao, Scott L. Crick, Caitlin L. Chicoine, Rohit V. Pappu.
Washington University in St. Louis, St. Louis, MO, USA.
Intrinsically disordered proteins (IDPs) adopt an ensemble of conformations under native physiological conditions. Despite their lack of folded structure, they perform important physiological functions and are predicted to constitute around 30% of the eukaryotic proteome. The success of disorder prediction using a protein's primary structure suggests that the propensity for disorder is encoded in the amino acid sequence. Recently, we found that net charge per residue segregates IDP sequences along a globule-to-coil transition, enabling construction of a sequence-space phase diagram that subdivides IDPs based on the polymeric character of their conformational ensembles. Here, we explore the effects of two perturbations that leave sequence composition unchanged: sequence permutation and pH titration. Using atomistic Monte Carlo simulations in ABSINTH implicit solvent, we find that permutants of an arginine-rich IDP sequence exhibit nearly identical polymeric attributes, yet differ in the details of their conformational ensembles. In contrast, fluorescence correlation spectroscopy shows a decrease in translational diffusion times with increasing pH, suggesting that electrostatic and conformational modulation of protonation equilibria is significant even for solvent-exposed titratable groups on flexible protein backbones. The effect is sufficient to induce a collapse transition in a polyarginine polypeptide at a pH well below the pKa of the arginine sidechain. We conclude that the polymeric character of an IDP is largely determined by its sequence composition, but emphasize the importance of accurate constant pH simulation technology in investigating details of its conformational equilibria.
Supported by grants 5T32GM008802 from the NIH National Institute of General Medical Sciences and MCB 0718924 from the National Science Foundation.

1333-Pos
Development of New Predictors of Intrinsically Disordered Proteins and Residues
Bin Xue[1,2], Christopher J. Oldfield[1], Weilun Hsu[1], Vladimir Uversky[1,2], A. Keith Dunker[1].
[1]Center for Computational Biology and Bioinformatics, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Institute for Intrinsically Disordered Protein Research, Indiana University School of Medicine, Indianapolis, IN, USA.
The concepts of intrinsic disorder (ID) and intrinsically disordered proteins (IDP) are being increasingly accepted by the scientific community. Although without unique 3D structures under physiological conditions, IDPs play important roles in many crucial biological processes, such as signaling, recognition, and regulation. However, identification of ID residues and IDPs is still challenging. Experimental methods are both time consuming and expensive. Computational methods are frustrated by the modest accuracy, especially on boundary regions and on short disordered segments. In attempts to improve the prediction accuracy, we developed four new predictors: CDF-all, PONDR-FIT, SPA, and Chopper. All these predictors showed some improvements over previous methods. CDF-all and PONDR-FIT employed artificial neural networks to refine the prediction results of six individual predictors (PONDR-VLXT, PONDR-VL3, PONDR-VSL2, IUpred, FoldIndex, and TopIDP). CDF-all predicts the disordered status of the entire sequence, while PONDR-FIT gives the disorder tendency of each residue. SPA was specifically designed for short peptides by creating sequence ensembles and taking the average of PONDR-VLXT predictions over the ensemble. Chopper cuts the query sequence into short segments and applies SPA on each short segment. The final prediction of Chopper is a refined average of SPA. Since the development of the first disorder predictor in 1997, there are currently more than 50 predictors. Although new predictors are continuously coming, the prediction accuracy seems to be approaching a ceiling. Our approach as demonstrated by these four methods is to develop new prediction strategies. CDF-all and PONDR-FIT integrate several individual predictors. SPA and Chopper enable focus on regions giving lower accuracies. These new approaches each provide additional improvement over previous predictors, but none of these new approaches significantly surpasses the ceiling, which is estimated to be near 85% accuracy for two state, structure/disorder, predictions.

1334-Pos
Structural Disorder Within Henipavirus Nucleoprotein and Phosphoprotein
Sonia Longhi, Johnny Habchi, Laurent Mamelli, Hervé Darbon.
AFMB, UMR 6098, CNRS and Universities Aix-Marseille I and II, Marseille, France.
Henipaviruses are newly emerged viruses within the Paramyxoviridae family. Their negative-strand RNA genome is packaged by the nucleoprotein (N) within a helical nucleocapsid that recruits the polymerase complex made of the L protein and the phosphoprotein (P). Using both computational and experimental approaches we herein show that Henipaviruses N and P proteins possess large intrinsically disordered regions. By combining several disorder prediction methods, we show that the N-terminal domain of P (PNT) and the C-terminal domain of N (NTAIL) are both mostly disordered, although they contain short order-prone segments. We then report the cloning, the bacterial expression, purification and characterization of Henipavirus PNT and NTAIL domains. By combining gel filtration, circular dichroism and nuclear magnetic resonance, we show that both NTAIL and PNT belong to the premolten globule sub-family within the class of intrinsically disordered proteins.

1335-Pos
Searching for the Native Molten Globules
Vladimir N. Uversky, A. Keith Dunker, Bin Xue, Christopher J. Oldfield.
Indiana University School of Medicine, Indianapolis, IN, USA.
Many biologically active proteins are either completely disordered or contain disordered regions of substantial size. These proteins are known as intrinsically disordered proteins, IDPs, among different names. The flexibility of these proteins and regions serves as the basis for their biological functions, where they are often involved in protein-protein interaction, regulation, recognition and signal transduction. These proteins are common in nature and are frequently associated with the pathogenesis of various human diseases Furthermore, intrinsic disorder-based protein-protein interactions, which are commonly accompanied by the disorder-to-order transitions, represent very attractive targets for novel drug development aiming at the specific inhibition of disease-associated protein-protein interactions. Therefore, it not surprising that IDPs have recently gained considerable attention. It is recognized now that ID comes in several flavors and that IDPs show an extremely wide diversity of their structural properties. The major pitfall of the current studies on IDPs in general and on the inhibition of the IDP-based protein-protein interactions in particular is that they are mostly focused on the extended IDPs almost completely ignoring a very substantial subset of IDPs, native molten globules. The goal now is to fill this gap and to identify and structurally and functionally characterize a set of native molten globules. The hypotheses that will be tested are the following: (i) native molten globules are very common in nature; (ii) native molten globules possess recognizable structural and functional properties and therefore can be found computationally and experimentally; (iii) native molten globules are frequently associated with human diseases.

1336-Pos
Exploring the Binding Diversity of Intrinsically Disordered Proteins
Wei-Lun Hsu.
Indiana University School of Medicine, Indianapolis, IN, USA.
Intrinsically disorder proteins often perform their functions through the binding of a short, loosely structured region from one protein onto the binding site of a protein partner. These short binding regions go by various names including eukaryotic linear motifs (ELMs), short linear motifs (SLiMs) and molecular recognition features (MoRFs). All of these represent a class of disordered protein that executes molecular recognition and binding functions typically via a disorder-to-order structural transition. Previous studies from our group showed 2 distinct examples of hub proteins, a disordered hub example (p53) and an ordered hub example (14-3-3), performing one-to-many signaling and many-to-one signaling, respectively. In the former, a single disordered region binds to multiple structured partners. In the latter, many distinct disordered regions bind to a single structured partner. Both alternatives use the MoRF mechanism described above. In this study, we tried to expand our previous work to find more examples and to determine whether the prevalence of the MoRF mechanism is common or rare through analyzing protein complexes deposited in PDB that consist of short nonglobular fragments bound to large globular partners. After examining and clustering the various MoRFs, we found 298 disordered hub examples and 246 ordered hub examples. Further experiments are providing detailed information about how intrinsic disorder facilitates binding to diverse partners. Exploring these examples is yielding a much clearer picture of the conformational changes that occur upon binding and showing that, in general, flexibility allows both subtle and complex structural variation thereby enabling different sequences to fit into the same binding site and the same