Editorial Overview

Intrinsically Disordered Proteins: Structure, Function and Therapeutics

Over the past two decades, the prevalence of intrinsically disordered proteins (IDPs) in biology has become broadly appreciated, which has been paralleled with understanding of the mechanisms that mediate their diverse functions. IDPs often exist as heterogeneous ensembles of fluctuating structures under physiological conditions, which offer advantages for many cellular functions. In particular, the inherent thermodynamic instability of IDPs could allow for their conformational properties to respond to numerous stimuli including binding to ligands or membrane surfaces, changes in the cellular environment, and post-translational modifications (PTMs). Multiple stimuli can readily be integrated through cooperative effects on their dynamic structural ensembles. These properties enable IDPs to fulfill the complex signaling and regulatory needs of higher organisms. As a consequence of their vital biological roles, IDPs are frequently associated with human diseases, including neurodegenerative diseases, numerous cancer types, and diabetes. This Special Issue contains five review articles [1–5] and 14 original research articles [6–19] that address some of the latest advances, central mechanistic questions, and emerging frontiers related to IDPs in biology and disease. In particular, the Issue provides physical insight into how intrinsic conformational disorder mediates diverse biological functions, so-called disorder–function relationships, and how this knowledge can be leveraged to therapeutically target IDPs in human disease.

A major challenge in establishing disorder–function relationships for IDPs is that their heterogeneous conformations are often incompletely characterized due to use of experimental methods that provide only ensemble-averaged structural parameters. This limitation can be addressed using computational methods, but further improvement of energy force fields as well sampling methodologies is required to generate accurate and well-converged structural ensembles that reflect experimental data. Furthermore, experimental approaches such as single-molecule methods and scattering techniques, which provide information on the distributions of conformers within ensembles rather than just average properties, are increasingly being used in studies of IDPs both *in vitro* and, in the case of single-molecule fluorescence methods, in living cells. In three original articles, Henriques *et al.* [6], Cragnell *et al.* [7], and Zheng and Best [8] describe new theoretical and computational approaches that utilize data from small-angle X-ray (or neutron) scattering experiments, which define the maximum dimensions of disordered protein conformations, to generate accurate IDP ensembles. Holmstrom *et al.* [9] report an application of state-of-the-art single-molecule Förster resonance energy transfer techniques to elucidate how structural plasticity of the hepatitis C virus core protein facilitates the formation of nucleocapsid-like particles at various viral life cycle stages. Many proteins contain both folded domains and disordered regions, and a continuing challenge is to understand how synergy between these fundamental elements mediates protein function. Combining an atomistic implicit solvent force field and an enhanced sampling method, Mittal *et al.* [10] map the sequence–conformation relationships of intrinsically disordered regions (IDRs) when tethered to folded domains and reveal how the presence of folded domains may modulate the conformational preferences of IDRs. An additional original research article, by Lin *et al.* [11], further illustrates how multi-scale modeling and simulation can be integrated with data from small-angle X-ray (or neutron) scattering and single-molecule Förster resonance energy transfer to provide insights into how disordered conformational ensembles are modulated by PTMs such as multi-site phosphorylation.

An important goal of structural studies of IDPs is to elucidate the physical mechanisms that underlie the interactions that mediate their functions in cells and how these are regulated. For example, the extent of disorder within certain IDRs is finely tuned to modulate affinity for functional partners [20]. In new studies, Poosapati *et al.* [12] examine how the residual structure of an IDP affects its interaction using the c-Myb transactivation domain as a model system. The review article by Berlow *et al.* [1] illustrates mechanisms through which intrinsic disorder enables allosteric regulation. The original research article by Beier *et al.* [13] further suggests that allosteric regulation may be achieved through modulation of correlated protein dynamics without formation of stable complexes between α-Synuclein and Calmodulin, and Osteopontin, and Heparin. These are just two of many examples wherein IDPs bind specifically to partners without folding into stable structures. A review article by Fuxreiter [2] provides a historical perspective on how "fuzziness" underlies the formation and regulation of

dynamic and heterogenous protein assemblies in cells. Entending this concept, the complexity and diversity of IDP functions and regulatory mechanisms is further showcased in the review article by Snead and Stochowiak [3], which discusses a new structure-independent mechanism of membrane fission that is driven by steric pressure generated by stochastic collisions among crowded, membrane-bound proteins, especially IDPs.

Bioinformatics analyses, starting in the late 1990s, established the prevalence of IDPs and IDRs in biology and annotated their diverse cellular functions, and continue to be powerful tools for large-scale analysis of proteomes to uncover novel signaling and regulatory mechanisms involving IDPs. These capabilities are exemplified by two original research articles in this Special Issue. First, Zhou *et al*. [14] examine functional relationships between sites of alternative splicing (AS) and PTMs, which are frequently co-located in IDRs. The results suggest that widespread synergy between protein disorder, AS, and/or PTMs contributes to complex cellular signaling in eukaryotic organisms. In addition, Mishra *et al*. [15] deploy a set of computational tools to analyze molecular recognition features in both structural and non-structural proteins of Zika virus, providing a basis for investigating the virus protein–host protein interaction network.

As participants in major disease pathways, many IDPs are potential drug targets. The ability to modulate the interactions of IDPs offers tremendous opportunities in chemical biology and molecular therapeutics. Targeting these proteins, which lack defined structures, requires detailed understanding of how intrinsic disorder contributes to disease pathogenesis and development of new small molecule screening and lead optimization strategies. This Special Issue contains two original research articles that address the oligomerization and aggregation of IDPs involved in neurodegenerative diseases. Williams *et al*. [16] present evidence for a novel mechanism wherein β-Synuclein engages in transient, multi-valent interactions with α-Synuclein to inhibit its aggregation. Also, Bhasne *et al*. [17] studied the molecular mechanism of α-Synuclein-tau heterotypic assembly and demonstrate that electrostatic interactions between these two proteins promote misfolding and accelerate aggregate formation. These mechanistic insights provide new directions for strategies to inhibit the aggregation of these neurodegeneration associated proteins. The formidable challenge of targeting IDPs is summarized in a review article by Tsafou *et al*. [4], which provides a synopsis of recent progress in targeting intrinsically disordered transcription factors. These advances, albeit limited, suggest that IDPs such as transcription factors are not undruggable as commonly thought [21]. Continual development of experimental and computational techniques for structural and functional interrogation of IDPs, as well as deeper understanding of their roles in biology and diseases, will provide the necessary foundation for overcoming the challenges associated with targeting IDPs. In addition, new conceptual frameworks are needed to develop small molecules or bio-therapeutics that modulate IDP structure and function in disease. Some of these questions are posed in the perspective article by Heller *et al*. [5], which presents a "structural ensemble modulation" framework for understanding IDP–small molecule interactions.

While some skeptics remain, the concept that protein disorder mediates diverse biological functions is firmly established. However, we often lack detailed mechanistic knowledge regarding how disorder confers function and, importantly, how the normal functions of IDPs and IDRs are altered in human disease. Together, the articles in this Special Issue represent the state-of-the-art regarding the biological roles of IDPs and IDRs, the mechanisms that mediate their diverse functions, and the potential for therapeutically targeting them in the future. We hope that this collection will inspire future studies of IDPs and IDRs by scientists of all ages from around the world, to enhance our knowledge of living systems and to improve the human condition.

References

[1] R.B. Berlow, H.J. Dyson, P.E. Wright, Expanding the paradigm: intrinsically disordered proteins and allosteric regulation, 2018.
[2] M. Fuxreiter, Fuzziness in Protein interactions—a historical perspective, 2018.
[3] W. Snead, J. Stachowiak, Structure versus stochasticity—the role of molecular crowding and intrinsic disorder in membrane fission, 2018.
[4] K. Tsafou, P.B. Tiwari, J.D. Forman-Kay, S.J. Metallo, J.A. Toretsky, Targeting intrinsically disordered transcription factors: changing the paradigm, 2018.
[5] G. Heller, M. Bonomi, M. Vendruscolo, A structural ensemble modulation mechanism of small molecule binding to disordered proteins, 2018.
[6] J. Henriques, L. Arleth, K. Lindorff-Larsen, M. Skepã, On the calculation of SAXS profiles of folded and intrinsically disordered proteins from computer simulations, 2018.
[7] C. Cragnell, E. Rieloff, M. Skepã, M. Skepã, Utilizing coarse-grained modelling and Monte Carlo simulations to evaluate the conformational ensemble of intrinsically disordered proteins and regions, 2018.
[8] W. Zheng, R.B. Best, An extended Guinier analysis for intrinsically disordered proteins, 2018.
[9] E.D. Holmstrom, D. Nettels, B. Schuler, Conformational plasticity of hepatitis C virus core protein enables RNA-induced formation of nucleocapsid-like particles, 2018.
[10] A. Mittal, A.S. Holehouse, M.C. Cohan, R.V. Pappu, Sequence-to-conformation relationships of disordered regions tethered to folded domains of proteins, 2018.
[11] X. Lin, S. Roy, M.K. Jolly, F. Bocci, N.P. Schafer, M.-Y. Tsai, Y. Chen, Y. He, A. Grishaev, G. Rangarajan, H. Levine, J. Orban, J.N. Onuchic, PAGE4 and conformational switching: insights from molecular dynamics simulations and implications for prostate cancer, 2018.

[12] A. Poosapati, E. Gregory, W. Borcherds, G. Daughdrill, Changing the fractional helicity of c-Myb TAD over a broad range has minimal effects on the binding affinity to KIX, 2018.
[13] A. Beier, T.C. Schwarz, D. Kurzbach, G. Platzer, F. Tribuzio, R. Konrat, Modulation of correlated segment fluctuations in IDPs upon complex formation as an allosteric regulatory mechanism, 2018.
[14] J. Zhou, S. Zhao, A.K. Dunker, Intrinsically disordered proteins link alternative splicing and post-translational modifications to complex cell signaling and regulation, 2018.
[15] P.M. Mishra, V.N. Uversky, R. Giri, Molecular recognition features in Zika virus proteome, 2018.
[16] J.K. Williams, X. Yang, T.B. Atieh, M.P. Olson, S.D. Khare, J. Baum, Multi-pronged interactions underlie inhibition of α-synuclein aggregation by β-synuclein, 2018.
[17] K. Bhasne, S. Sebastian, N. Jain, S. Mukhopadhyay, Synergistic amyloid switch triggered by early heterotypic oligomerization of intrinsically disordered α-synuclein and Tau, 2018.
[18] L.D.T. Kwan, J.J. Hollins, J. Clarke, Promiscuous and selective: how intrinsically disordered BH3-proteins interact with their pro-survival partner MCL-1, 2018.
[19] C. Bignon, F. Troilo, S. Gianni, S. Longhi, Partner-mediated polymorphism of an intrinsically disordered protein, 2018.
[20] W. Borcherds, F.X. Theillet, A. Katzer, A. Finzel, K.M. Mishall, A.T. Powell, et al., Disorder and residual helicity alter p53–Mdm2 binding affinity and signaling in cells, Nat. Chem. Biol. 10 (2014) 1000–1002.
[21] A.N. Koehler, A complex task? Direct modulation of transcription factors with small molecules, Curr. Opin. Chem. Biol. 14 (2010) 331–340.

Jianhan Chen
Department of Chemistry, University of Massachusetts, Amherst, Amherst, MA 01003, United States
Department of Biochemistry & Molecular Biology, University of Massachusetts, Amherst, Amherst, MA 01003, United States
Corresponding author. Department of Chemistry, University of Massachusetts, Amherst, Amherst, MA 01003, United States.
E-mail address: jianhanc@umass.edu.

Richard W. Kriwacki
Department of Structural Biology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States
Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Sciences Center, Memphis, TN 38105, United States
Corresponding author. Department of Structural Biology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States.
E-mail address: richard.kriwacki@stjude.org.