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Digested disorder

Quarterly intrinsic disorder digest (January/February/March, 2013)

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The current literature on intrinsically disordered proteins is blooming. A simple PubMed search for "intrinsically disordered protein OR natively unfolded protein" returns about 1,800 hits (as of June 17, 2013), with many papers published quite recently. To keep interested readers up to speed with this literature, we are starting a "Digested Disorder" project, which will encompass a series of reader's digest type of publications aiming at the objective representation of the research papers and reviews on intrinsically disordered proteins. The only two criteria for inclusion in this digest are the publication date (a paper should be published within the covered time frame) and topic (a paper should be dedicated to any aspect of protein intrinsic disorder). The current digest covers papers published during the period of January, February and March of 2013. The papers are grouped hierarchically by topics they cover, and for each of the included paper a short description is given on its major findings.

Introduction

The protein intrinsic disorder phenomenon is becoming a popular research topic. This bold statement is illustrated by **Figure 1** which represents the results of a very simple PubMed search for just two terms, "intrinsically disordered protein" OR "natively unfolded protein," which returned almost 1,800 hits (as of June 17, 2013). **Figure 1** shows that the number of protein disorder-related publications increases exponentially and that the number of papers published in 2012 only reaches 391 counts, clearly exceeding the one-paper-per-day limit. Obviously, the number of papers derived via this simple PubMed search represents a lower limit of protein disorder-related studies, since many more papers are dedicated to proteins whose intrinsically disordered nature is known but this fact is not mentioned in the corresponding study. For example, PubMed search for "(intrinsically disordered) OR (natively unfolded) AND synuclein" returned 172 hits, whereas there are 5,405 papers in PubMed which contain "synuclein" in their texts.

Since the field is developing fast and since a number of important observations related to protein intrinsic disorder are now reported on a daily basis, a "Digested Disorder" project is started by the *Intrinsically Disordered Proteins* journal. The goal of this project is to keep the interested readers updated on the recent developments in the field by providing a reader's digest-kind of articles which would represent objective overviews of the research papers and reviews on intrinsically disordered proteins published during the specified period. It is planned that the Digested Disorder articles will appear at the end of each published issue. No specific filtering will be used and any PubMed annotated paper dealing with the protein intrinsic disorder phenomenon and published during the period covered will be included in the digest. The digest article is structured hierarchically and papers are grouped in several sections: (1) structures of intrinsically disordered proteins (IDPs); (2) functions of IDPs; (3) methods for the IDP analysis; (4) proteomics of IDPs; (5) IDPs and diseases; and (6) IDPs/IDPRs as drugs or drug targets. One should keep in mind that the unambiguous classification of many papers is challenged by the intertwining of topics they cover.

Studies covered by the first paper of this series are research papers and reviews published during the period of January, February and March of 2013. The target papers were found by the PubMed search engine using the advanced search tool with the query containing the following terms "((intrinsically disordered protein) OR (natively unfolded protein)) AND ("2013/01/01" [Date - Publication]: "2013/03/31" [Date - Publication])." The retrieved 114 papers were further analyzed to make sure that they are published within the specified period and that they are indeed dealing with the IDPs or IDP regions (IDPRs). Any publications that did not satisfy the chosen search criteria were removed.

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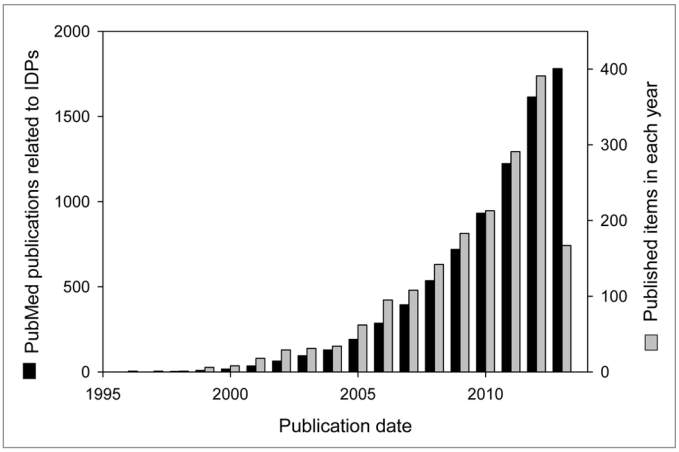

Figure 1. An exponential increase in number of papers dealing with intrinsically disordered proteins. The plot represents the results of a very simple PubMed search where just two terms, "intrinsically disordered protein" OR "natively unfolded protein," were used. This search returned almost 1,800 hits (as of June 17, 2013). The actual number of publications dealing with intrinsically disordered proteins is essentially greater.

Studies on Structural Properties of IDPs and IDPRs

IDPs/IDP regions are different from ordered proteins and domains not only at the level of their amino acid sequences, but at higher levels of structural organization too, possessing a wide array of characteristic and recognizable structural properties.[1] For example, their conformational ensembles contain highly dynamic structures that interconvert on a number of timescales.[2] Although in early studies it was emphasized that IDPs/IDP regions could be crudely grouped into two major classes, proteins with extended and compact disorder,[2-7] it is recognized now that IDPs/IDPRs are characterized by extreme sequential, structural, and spatiotemporal heterogeneity of IDPs; the rough and relatively flat energy landscapes; the ability to undergo both induced folding and induced unfolding; the ability to interact specifically with structurally unrelated partners; the ability to gain different structures at binding to different partners; and the ability to keep essential amount of disorder even in the bound form.[8] In fact, currently available data suggest that intrinsic disorder can have multiple faces, can affect different levels of protein structural organization, and whole proteins, or various protein regions can be disordered to a different degree.[8,9] According to this classification, proteins can contain more or less of intrinsic disorder, and IDPs/IDPRs can be less or more compact, possess smaller or larger amounts of flexible secondary structure and contain smaller or larger numbers of tertiary contacts. Papers represented in this section are about various structural aspects and conformational behavior of IDPs and IDPRs.

Since neither IDPs nor IDPRs are able to fold autonomously into specific structures, their structural description requires conformational ensemble-based approaches. Mao et al. evaluated recent advances in quantifying sequence-ensemble relationships achieved through a four-way synergy between bioinformatics, biophysical experiments, computer simulations and polymer physics theories.[10] The authors concluded that understanding of the quantitative relationships between information encoded in the amino acid sequences of disordered proteins and the ensemble of conformations they sample is crucial for the development of quantitative models able to unambiguously describe the sequence-ensemble relationships of intrinsically disordered proteins.[10]

Tantos et al. performed a comprehensive structural characterization of an interesting highly phosphorylated IDP, human nucleolar phosphoprotein p140 (hNopp 140).[11] The authors utilized a wide spectrum of techniques, such as anomalous electrophoretic mobility, protease sensitivity, heat stability, hydrodynamic behavior on size-exclusion chromatography, wide-line NMR analysis, ^{1}H-NMR spectroscopy, differential scanning calorimetry, circular dischroism spectroscopic analysis under the variety of conditions, and partner binding. According to this multiparametric analysis, hNopp 140 a 699 residues-long protein that has ~20 phosphorylated residues and serves as protein inhibitor of casein kinase 2 (CK2), was shown to behave as native pre-molten globule with a noticeable predisposition to become ordered in the presence of its binding partner.[11]

Marsini et al. used a small-angle X-ray scattering (SAXS)-based ensemble optimization method to analyze the effect of phosphorylation on structural properties of the regulatory domain (RD) of the cystic fibrosis transmembrane conductance regulator (CFTR), which acts as an anion channel activated by protein kinase A phosphorylation in the norm and which is compromised in cystic fibrosis.[12] SAXS analysis revealed that phosphorylation at multiple sites induces noticeable compaction of this intrinsically disordered domain. These SAXS data were used in an ensemble optimization method to build the first experiment-based, low-resolution 3D-models of the native and the phosphorylated RD.[12]

Based on the computational and experimental analyses, Elam et al. concluded that temperature and urea possess opposing impacts on polyproline II (PII) conformational bias.[13] In fact, the predicted and experimentally observed PII propensity was shown to decrease with an increase in temperature, whereas urea promoted the PII conformation.[13]

Mizuguchi and Okazawa reviewed currently available information on structural properties of the polyglutamine tract-binding protein 1 (PQBP1).[14] PQBP1 is an intrinsically disordered nuclear protein regulating pre-mRNA splicing and transcription, mutations in which are associated with hereditary mental retardation. Although the ^{1}H-^{15}N HSQC NMR spectra of the polar domain and the C-terminal domain of PQBP1 are typical of highly disordered proteins, a continuous 23-residue segment of the C-terminal domain is used in binding to target protein U5–15 kD.[14]

Analyzing Functions of IDPs and IDPRs

The extreme conformational plasticity of IDPs and IDPRs is crucial for their intricate and multifarious biological roles. Functions of IDPs and IDPRs complement functional repertoire of ordered proteins and domains, with disordered proteins being commonly involved in a wide range of intermolecular interactions.[15-17] Furthermore, because sites within their polypeptide chains are highly accessible, IDPs can undergo extensive post-translational modifications (PTMs), such as phosphorylation, acetylation, and/or ubiquitination (sumoylation, etc.,), allowing for modulation of their biological activity or function.[18,19] Many IDPs and IDPRs are known to undergo complete or partial disorder-to-order transition as a result of interaction with specific binding partner(s).[20,21] Some IDPRs possess remarkable binding promiscuity, being capable of specific interactions with many structurally unrelated partners.[22] IDPRs are also able to gain different structures at binding to different partners,[22,23] and many IDPs/IDPRs are characterized by the ability to keep essential amount of disorder even in their bound forms.[8,24-26] Inside the cell, the abundance and functionality of IDPs, these crucial regulators of almost all cellular processes, are under tight surveillance.[27,28] However, although many IDPs were shown to be less abundant than ordered proteins due to the lower rate of protein synthesis and shorter protein half-lives, some IDPs are known to be present in cells in large amounts or/and for long periods of time due to either specific PTMs or via interactions with other factors, which could promote changes in cellular localization of IDPs or protect them from the degradation machinery.[4,19,29-31] Papers collected in this section are dedicated to the various functional aspects of IDPs and IDPRs.

An interesting discussion eliminating two opposite viewpoints on the phenomenon of protein intrinsic disorder was represented in published side-by-side papers by Uversky and Dunker[32] and Janin and Sternberg.[33] Uversky and Dunker argued that protein intrinsic disorder represents a novel and useful concept that helps better understanding protein functionality and correlation between protein structure and function.[32] Janin and Sternberg took an opposing viewpoint, arguing that flexibility, not disorder, is an intrinsic property of proteins, and that most IDPs are in fact proteins waiting for a partner (PWPs), which serve as parts of a multi-component complex that do not fold correctly in the absence of other components.[33]

Ubiquitination represents an important step in regulating functions of some proteins and in defining fate of other proteins. One of the best known applications of protein ubiquitination is the highly specific targeting of substrates for proteasomal degradation. Ubiquitination requires the participation of at least three different enzymes, a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). To understand the determinants for the site-specific ubiquitination by E3 ligase, Landre et al. studied the roles of the E3 ligase docking site located within an intrinsically disordered Mf2 domain of the interferon regulatory factor-1 (IRF-1) in ubiquitination of this short-lived interferon γ-regulated transcription factor.[34] The analysis revealed that the IRF-1 ubiquitination happened predominantly at the lysine residues located within loops protruding from the DNA-binding domain, whereas the highly disordered C-terminal half of the protein was not modified at all. Furthermore, the efficiency of the IRF-1 ubiquitination was shown to be modulated by binding of this transcription factor to DNA, suggesting the DNA-bound pool of IRF-1 is functionally protected from polyubiquitination and degradation.[34]

A phenomenological model was proposed by Das et al. to explain the N-type inactivation of potassium channels by their intrinsically disordered cytosolic loops.[35] This model suggested the N-type inactivation through disordered regions, which is known to be stereospecific and dependent on the channel type, represents an illustrative example of the disorder-based function through conformational selection and not vs. induced fit. Importantly, this study also suggested that some subtle changes in the amino acid sequences of disordered regions represent crucial modulating factors defining binding efficiency and specificity in the absence of folding.[35]

A comprehensive review by Kovacs et al. is dedicated to the analysis of various mechanisms of the binding-induced folding of IDPs and to the elucidation of the roles of IDPs in assisting folding of other proteins.[36] Among subject covered in this important review are molecular mechanisms of IDP binding, such as induced folding and conformational selection; the use of short motifs and large disordered domains in recognition by IDPs; folding of IDPs/IDPRs before and after binding; binding of IDPs/IDPRs without folding (fuzziness).[36] The authors also analyzed the interplay between protein disorder in chaperones and the assistance of folding of other protein. Among subjects covered there are molecular mechanisms of chaperone action, description of chaperone machines with IDPRs, fully disordered chaperones, late embryogenesis abundant proteins, RNA chaperones, and action of disordered chaperones via mutual induced folding transitions.[36] Finally, the various roles of intrinsic disorder in protein quality control system were considered, with major focus being at the ubiquitin–proteasome system and its components, such as E3 ubiquitin ligases, as well as at the role of structural disorder in substrate recognition function of chaperones.[36]

The topics of the intrinsic disorder-based protein interactions and various modes of their modulation, regulation and control are continued in a review by Uversky.[37] Here, various interaction modes of IDPs/IDPRs are compared with the interaction mechanisms of ordered proteins and domains. Considered binding models of ordered proteins include a classic "lock-and-key" model, "induced fit" hypothesis, "conformational selection" mechanism, extended "conformational selection" model (where conformational selection is followed by conformational adjustments), and the "game theory" model.[37] On the other hand, intrinsic disorder-based interactions can result in the formation of various static and dynamic (fuzzy) complexes via several mechanisms, such as binding-induced folding, conformational preference, fly-casting, and game theory for static complexes, and

polyelectrostatic model, binding cloud, and halted encounter complex formation model for the fuzzy complexes. A multitude of protein disorder-based interactions modes (e.g., molecular recognition features (MoRFs), flexible wrappers, penetrators, huggers, intertwined strings, etc.,) is introduced, together with the molecular "anatomy" and "physiology" of interactions mediated by IDPs and some means for regulation of the disorder-based interactions (such as functional regulation via PTMs and alternative splicing).[37]

Barbour et al. analyzed the peculiarities of the ubiquitin-independent proteasomal degradation of human thymidylate synthase (hTS) mediated by an N-terminally located degron.[38] hTS degron consists of two subdomains, a highly divergent IDPR and a conserved amphipathic α-helix, and possesses a unique capability to destabilize the heterologous proteins to which it is attached. The authors also found a cryptic degron at the C-terminus of hTS that function only under certain circumstances and the activation of which is controlled by the N-terminal degron.[38]

Dube et al. reported on the discovery of two novel genes in cold-acclimated crowns of alfalfa (*Medicago sativa* spp sativa L.) encoding intrinsically disordered dehydrins that have potential adaptive value with regard to the freeze-induced cell dehydration and therefore play important roles in tolerance of this plant to subfreezing temperatures.[39]

Wang et al. established that a small calmodulin (CaM)-binding IDP, PEP-19, that is expressed in cells exhibiting highly active Ca^{2+} dynamics, can bind Ca^{2+} by itself, thereby modulating Ca^{2+} binding to CaM.[40] PEP-19 was also able to influence the ATP-dependent Ca^{2+} release in HeLa cells, with mutations affecting its Ca^{2+} binding efficiency dramatically inhibiting the PEP-19 effects on ATP-induced Ca^{2+} release. Based on these observations the authors concluded that PEP-19 is placed at the top of the CaM signaling cascades, and that its cellular effects depend both on CaM binding and on the ability to interact with Ca^{2+}, thereby expanding the biological significance of this small IDP.[40]

Based on the structural, dynamic, and functional analysis of an intrinsically disordered domain of the sulfhydryl oxidase ALR (augmenter of liver regeneration) located in the intermembrane space of mitochondria, Banci et al. concluded that this disordered domain has a dual function in two cellular compartments, acting as a mitochondrial targeting signal in the cytosol and serving as a crucial recognition site in the disulfide relay system of intermembrane space.[41] Therefore, the intrinsically disordered domain of the sulfhydryl oxidase ALR represents an illustrative example of a moonlighting IDP with two distinct functions linked to intracellular organelle targeting.[41]

Bansal et al. uncovered an intricate mechanism of the formation of functional SLIP1-SLBP complex that activates translation of replication-dependent histone mRNAs.[42] This study revealed that the "active" SLIP1-SLBP complex is formed only when phosphorylated intrinsically disordered human SLBP first binds to the histone mRNA stem-loop motif and then recruits SLIP1. Intriguingly, the non-phosphorylated SLBP has a weak affinity for SLIP1. Another curious observation is the fact that although non-phosphorylated SLBP is engaged in the formation of a high affinity SLIP1-SLBP heterotetramer, this complex is incapable of the mRNA binding.[42] Therefore, phosphorylation of the intrinsically disordered SLBP plays a crucial role in the formation and activity of the SLIP1-SLBP complex.[42]

In agreement with the idea that IDPs might possess a wide spectrum of binding affinities, Drobnak et al. showed that an IDP can achieve very strong and structurally well-defined binding resulting in the formation of highly ordered complex.[43] This conclusion is based on a comprehensive thermodynamic and structural analysis of the binding of an intrinsically disordered CcdA to gyrase poison CcdB. This interaction is characterized by the binding affinity up to the picomolar range and leads to the complete folding of CcdA. The CcdB bound form of this originally disordered protein is well-folded, being stabilized mostly by specific intramolecular interactions, suggesting that the folded structure is defined largely by CcdA own amino acid sequence, with the binding partner functioning as a facilitator of folding than a template.[43]

Study by Khan et al. provides a strong support to the fuzzy complex concept via a detailed characterization of an interaction between the typical IDP, prothymosin α, and Kelch domain of Kelch-like ECH-associated protein 1 (Keap1).[44] This interaction was characterized by nuclear magnetic resonance spectroscopy, isothermal titration calorimetry, peptide array analysis, site-directed mutagenesis, and molecular dynamic simulations. The analysis revealed that prothymosin α retains a high level of flexibility, even in the bound state with Kelch. Furthermore, based on the mutational analysis of prothymosin α, guided by peptide array data and isothermal titration calorimetry, the prothymosin α region $_{38}$NANEENGE$_{45}$ was shown as a primary binding site for the Kelch domain of Keap1.[44]

Huang et al. investigated regulation of the interactions between the cytoplasmic poly(A)-binding protein C1 (PABPC1) and the PAM2 motif-containing proteins.[45] *In silico* analysis revealed that PAM2 motifs are embedded within IDPRs next to next to cluster(s) of potential phosphorylation sites, suggesting that phosphorylation at these sites is needed for regulation of the interactions between PAM2-containing proteins and PABPC1. This hypothesis was validated via the analysis of the effect of variable phosphorylation on the PABPC1 binding affinity of three PAM2-containing proteins (Tob2, Pan3, and Tnrc6c).[45]

An interesting mechanism of phosphorylation-induced regulation of a hybrid protein containing ordered and intrinsically disordered domains was described by Chen et al.[46] These authors analyzed an F-actin binding and bundling protein dematin (band 4.9) and showed that phosphorylation of the well folded C-terminal villin-type headpiece of this protein increased the affinity of this structured module to the intrinsically disordered N-terminal core domain of dematin leading to the formation of a compact structure that sterically eliminated one of the F-actin binding sites, thereby reducing the F-actin bundling activity of this important regulatory protein.[46]

Kjaergaard et al. analyzed one illustrative example of morphing MoRFs; i.e., disorder-based binding regions with

remarkable adaptability that can fold into different conformations depending on the binding partner.[47] The authors analyzed the millisecond dynamics of the nuclear coactivator binding domain of CBP (NCBD) using relaxation dispersion NMR spectroscopy and showed that the energy landscape of this domain resembles the energy landscapes of the fold-switching proteins that have two coexisting native states. Such a configuration of the energy landscape may serve as an explanation for binding via conformational selection.[47]

Hayward et al. analyzed the functional roles of IDPR and ordered domain in the product of the mitochondrial genome maintenance gene, MGM101.[48] To this end, they studied the chimeric Mgm101p proteins from the yeast *Saccharomyces cerevisiae* and the coral *Acropora millepora* with switched ordered core regions and disordered N-terminal domains. This analysis revealed that the ordered domain of *A. millepora* can functionally replace the yeast core region, whereas lost function of the disordered yeast N-terminal domain cannot be replaced by its coral counterpart.[48]

A new family of IDPs involved in stabilization of the bacterial carbon storage granules was described by Maestro et al.[49] One of these newly discovered IDPs is PhaF phasin from *Pseudomonas putida* KT2440. The intrinsically disorder nature of this protein was validated by a series of hydrodynamic, spectroscopic and thermodynamic experiments, whereas computational tolls suggested that other members of the phasin family are intrinsically disordered in the absence of its ligands.[49]

The roles of IDPRs in function of small- and intermediate-conductance Ca^{2+}-activated potassium (SK/IK) channels were investigated by Zhang et al.[50] The authors showed that although a fragment of SK channels that connects the transmembrane segment S6 and the clamodulin-binding domain is disordered (being invisible in the protein crystal structure), this IDPR becomes readily identifiable in the presence of the specific compound that potentiates the channel activities.[50] Furthermore, this compound-stabilized IDPR increases the channel open probability at a given Ca^{2+} concentration, emphasizing crucial biological role of this fragment in coupling Ca^{2+} sensing by calmodulin and mechanical opening of SK channels.[50]

Methods for the IDP/IDPR Analysis

Computational approaches for the analysis of intrinsic disorder. The fact that amino acid sequences of IDPs and IDPRs possess several recognizable features is well-established,[2,4,8,51-54] and various computational tools for finding intrinsic disorder in proteins are being developed with an amazing pace.[55-62] It was emphasized that the existence of numerous computational tools that give prediction of disorder far above that expected by chance provides direct support for the hypothesis that intrinsic disorder is encoded in a protein's amino acid sequence.[1] Papers below are dedicated to various computational tools elaborated for the analysis of IDPs and IDPRs.

Predictors of intrinsic disorder. Huang et al. proposed to use the Nearest Neighbor algorithm to find protein disordered regions.[63] As the encoding features of each amino acid in a fixed-length sliding window, this sequence-based computational approach uses an optimal 51-feature set that includes 39 conservation features and 12 secondary structure features selected based on mRMR (maximum Relevancy Minimum Redundancy). Since this new method was reasonably accurate, the authors concluded that sequence conservation and secondary structure might play various important roles in IDPs/IDPRs.[63]

Jin and Lui dedicated their study to the finding of the inherent relationships among different biophysics-based predictors of intrinsic disorder.[64] Based on the correlation analysis approach of realistic data sets, they found correlations among some physical-chemical properties (charge-hydropathy plot, packing density, pair-wise energy) typically used for protein disorder predictions. This allowed determination of a projected direction to discriminate ordered and disordered proteins.[64]

Molecular dynamics simulations. Molecular dynamics (MD) simulations are ideally suited to investigate protein and peptide plasticity and flexibility simultaneously at high spatial (atomic) and high time resolution.[65] Despite limitations determined by the force field accuracy and by the maximum simulation time that can be routinely achieved in current MD simulations, this technique provides a first principle-based description of the conformational behavior of proteins.

Mittal et al. performed replica exchange molecular dynamics simulation of an intrinsically disordered 15-residue wild-type p53 fragment from the TAD domain and its mutant (TAD-P27L) using an optimized (fully atomistic, explicit solvent) protein model and the experimental validation of the simulation results.[66] This analysis revealed that although this peptide is characterized by the relatively flat conformational free-energy landscape, its conformational ensemble contains significant fraction of solution structures resembling the MDM2-bound form.[66]

McDowell et al. performed extensive atomistic simulations of the $S100B_{\beta\beta}$-bound conformation of p53 negative regulatory domain (NRD) in explicit solvent (with 1.0 μs total effective sampling).[67] In agreement with earlier NMR studies, this analysis revealed that p53-NRD preserves significant flexibility when bound to $S100B_{\beta\beta}$, providing an atomistic description of this important fuzzy complex.[67]

Computational analysis of IDP structures and functions. One of the important features of disordered protein binders is their ability to fold differently as a result of interaction with different binding partners. Originally, this concept of binding diversity and divergent binding-induced folding was introduced based on the example provided by the C-terminal regulatory domain of p53, the same short segment of which interacts with several structurally unrelated partners adopting different conformations (an α-helix, a β-sheet, and differently laid irregular structures) when bound to the different partners.[22] Hsu et al. continued analysis of this interesting phenomenon of the MoRF-based one-to-many protein-protein interactions, where MoRFs represent short IDRPs that bind to partners via disorder-to-order transitions.[23] To this end, a set of multispecific MoRFs (i.e., MoRFs that were shown to bind to 2–9 structurally dissimilar

partners) was carefully selected from PDB and analyzed. This analysis confirmed that a multispecific MoRF uses different residues to interact with different partners and showed that binding diversity is further promoted by alternative splicing events (ASEs) and PTMs. Overall, this suggested that MoRFs, ASEs, and PTMs may collaborate to differently alter protein-protein interaction networks in different cell types and all contribute to the evolvability of signaling network diversity.[23]

Krzeminski et al. introduced the latest version of a useful computational approach, ENSEMBLE, that allows determination of a set of conformations that represents the structural ensemble of a disordered protein based on input experimental data.[68] This new ENSEMBLE is further enhanced by inclusion of an intuitive user interface and some new approaches that ameliorated data treatment and result analysis.[68]

Matsushita and Kikuchi looked at the potential functional consequences of structural frustration and at the correlation between structural frustration and protein disorder.[69] In a model of protein folding based on the spin glass theory,[70] the spontaneous folding of an ordered protein is determined by the minimal frustration principle, which defines shape of the funnel energy landscape.[71] The minimal frustration principle also predicts that strong frustration makes the energy landscape rugged,[70] suggesting that frustration only leads to protein functional disorder.[69] However, IDPs and IDPRs, with their highly flexible structures, are characterized by rugged energy landscapes, suggesting that their behavior is not driven by the minimal frustration principle. The authors extended the Wako-Saitô-Munoz-Eaton model to include a consideration of the frustration effects and showed that designed structural frustration induces intrinsic disorder in a protein.[69] Furthermore, frustrated structure was able to undergo cooperative folding as a result of binding to a target protein, providing an IDP with physical means to exhibit a sharp switch-like folding response to binding. Based on these observations the authors concluded that the structural frustration may define switch-like functionality of IDPs.[69]

Experimental approaches for the analysis of intrinsic disorder. Obviously, structural characterization of highly heterogeneous ensembles of IDPs/IDPRs requires rather specialized approaches. In fact, the determination of a unique high-resolution structure is not possible for an isolated IDP, and rather complex methods have to be used to obtain experimental constraints on the ensemble of states that is sampled by the intrinsically disordered polypeptide chain. Therefore, IDP-related structural studies typically rely on a host of biophysical methods that can provide information on the overall compactness of IDPs, their conformational stability, shape, residual secondary structure, transient long-range contacts, regions of restricted or enhanced mobility, etc.[1] Currently, there are more than 70 experimental approaches for structural/conformational characterization of IDPs/IDPRs.[7,72-77] Papers below introduce various experimental approaches and their applications for the analysis of structures and functions of IDPs and IDPRs.

NMR. Ota et al. proposed a unique computational method to assign IDPRs based on NMR structures.[78] The tool was developed based on the comparison of missing residues of X-ray structures with residue-wise deviations of NMR structures for identical proteins. Based on this analysis, a threshold deviation of 3.2 Å was derived for the best correlation of ordered and disordered regions of both structures and then was applied for the analysis of proteins whose structures were only determined by NMR. The authors believe that this tool can significantly extend the current pool of proteins with experimentally determined IDPRs.[78]

Nyarko et al. proposed a new approach to identify active recognition motifs based on NMR-detected β-sheet propensities.[79] Furthermore, these authors analyzed the intrinsically disordered Dyn2 binding domain of Nup159 by solution NMR and isothermal titration calorimetryand showed that binding of one equivalent of Dyn2 dimer aligns two Nup159 chains along the full Dyn2 binding domain to form a bivalent scaffold that promotes binding of other Dyn2 dimers.[79]

An effective method for generation of the small bicelles with a uniform confined size that display a series of gangliosides was proposed by Yamaguchi et al.[80] These functionalized bicelles represent nanoscale standardized membrane mimics suitable for NMR characterization of weak encounter complexes formed between ganglioside clusters and amyloidogenic IDPs, such as α-synuclein.[80]

Prestegard et al. reported ^{1}H and ^{15}N chemical shifts for a set of 20 alanine based pentapeptides, with the central residue of each being varied among the 20 amino common acids, under the low pH urea denaturing conditions.[81] This new set of chemical shits is then used in empirical formula to predict chemical shifts of unfolded proteins, which is a crucial starting point in the process of the cross peak assignment in ^{1}H-^{15}N HSQC spectra of sparsely labeled proteins (including IDPs). The proposed in this study set of chemical shits is more appropriate for sparse label assignments, since the authors showed a small, but significant, improvement in shift predictions for unfolded ubiquitin.[81]

Since the quality of NMR spectra of IDPs is known to be lower than that of ordered proteins, being characterized by low chemical shift dispersion and efficient broadening (often beyond detection) of amide proton resonances, Bermel et al. discussed some key aspects that need to be taken into account when new NMR experiments optimized for the study of IDPs are designed and proposed one of such new experiments based on direct detection of $^{13}C_{\alpha}$.[82] Here, the authors emphasized that in the NMR-based analysis of IDPs it is important to exploit heteronuclei, since a prominent increase in the chemical shift dispersion was evident passing from ^{1}H to the directly bound heteronucleus, both for $^{13}C_{\alpha}$ as well as for ^{15}N. Also, the correlation of ^{15}N with the attached carbonyl carbon ($^{13}C'$) through the 2D CON spectrum represents another useful trick to further improve the cross peak dispersion and to detect signals deriving from proline residues.[82]

The topic of the improvement of NMR experiments for the analysis of IDPs was continued in the article by Kim et al. who investigated the effect of fast hydrogen exchange (HX) of unprotected amide protons with protons from the solvent on the measurement of the ^{15}N transverse

relaxation rate (R_2), which is crucial for the analysis of the protein backbone dynamics.[83] The authors used ^{15}N backbone transverse relaxation experiments (R_2) using the CPMG (Carr–Purcell–Meiboom–Gill) pulse train ($^{15}N\ R_2^{CPMG}$) to investigate dynamics of solvent exposed backbone amides in α-synuclein. This analysis revealed that although the R_2^{CPMG} rates are modulated by fast HX rates, the HX effect on R_2^{CPMG} can be extracted by the derived equation and therefore can be corrected quantitatively.[83]

Photo-crosslinking. An article by Phan et al. describes the use of various photo-crosslinking approaches for mapping the interactome networks inside the living cells.[84] At the first stage of a new generation of photo-crosslinking methods, the photo-crosslinking analogs of amino acids or sugars are incorporated into cellular biomolecules via the metabolic engineering or genetic code expansion. Then, the crosslinked complexes and related interactomes are analyzed by mass spectrometry and immunological techniques. This analysis, being performed under the conditions of living cell, provides a unique possibility of gaining information on the context-dependent interactions. Photo-crosslinking is well-suited for mapping interaction interfaces and determining the interactome dynamics. These techniques can be also used for finding and characterization of transient interactions typical for IDPs.[84] Some illustrative examples of the successful use of cell-based photo-crosslinking are given, where photo-crosslinking methods were apply to gain information on chaperone-assisted protein folding, transcription, nucleo-cytoplasmic transport, membrane protein dynamics, and immune cell signaling.[84]

Neira represented a comprehensive review of recent advances in the application of NMR for the structural analysis of viruses.[85] The author emphasized that NMR can be used for the determination of solution structures of viral proteins and their isolated domains (including viral IDPs and IDPRs), for the description of their conformational changes and complex dynamic equilibria, for building of pseudo-atomic models of entire virus capsids, and for the identification of conformational changes in intact viral capsids accompanying their insertion to the host membranes.[85]

EPR. Martinho et al. looked at the induced folding of the intrinsically disordered C-terminal domain of the nucleoproteins (N_{TAIL}) from the Nipah and Hendra viruses (which are two recently emerged pathogens gathered within the *Henipavirus* genus) by site-directed spin labeling coupled to electron paramagnetic resonance (EPR) spectroscopy (SDSL EPR).[86] This approach, that relies on the introduction of a paramagnetic spin label through covalent modification of a unique sulfhydryl group (e.g., of the strategically introduced cysteine residue) using a selective nitroxide reagent, and on the subsequent analysis of the EPR spectra whose shape reflects the mobility of the spin label, represents a very useful addition to the existing arsenal of the biophysical techniques for the structural characterization of IDPs/IDPRs.[87,88] Since cysteine residues can be introduced at any part of the protein sequence, SDSL EPR can, in principle, provide information at the residue level, while introducing minimal perturbation of the system. The authors showed that this technique can be used to establish structural differences between the homologous proteins.[86] For example, the portions of the N_{TAIL} proteins from the Nipah and Hendra viruses that are responsible for the interaction with the C-terminal X domain of the phosphoprotein (P_{XD}), possess noticeable structural differentiation, with the Nipah virus N_{TAIL} protein being characterized by the conformational heterogeneity of the partly preconfigured α-helix that contained some stable α-helical segments.[86]

High pressure studies. Somkuti et al. broadened the range of experimental conditions traditionally used for structural and conformational analysis of proteins by considering the effects of a wide range of pressure and temperature on the ordered and disordered domains of titin.[89] This was done using Fourier transform infrared (FTIR) and fluorescence spectroscopy combined with a diamond anvil cell that allowed investigation of protein secondary structure and fluorescent parameters across the broad range of pressure (0–16 kbar), temperature (0–100°C), pD (3–10.5), and different solvent conditions.[89] As targets proteins, they used an ordered Ig domain (I27) and a 171-residue-long fragment (polyE) of the disordered PEVK domain derived from titin,[89] a giant elastic protein (canonical form of human titin consists of 34,350 residues) responsible for striated-muscle elasticity, which is known to possess a lot of disorder.[90] This analysis revealed that the PolyE domain preserved its disordered characteristics in the whole range of conditions studied, whereas structured I27 possessed an intricate conformational response to changing conditions that can only be described by the complex temperature-pressure phase diagram.[89]

Mass spectrometry-based methods. The use of various mass spectrometry methods for the analysis of IDPs and IDPRs was covered by Beveridge et al. in a comprehensive review.[91] Here, the authors emphasized that the last decade witnessed revival of mass spectrometry as a structural tool, and this explosion in the use of various mass spectrometry methods was provoked by the intensive use of this powerful biophysical technique for obtaining unique insights into the structure and dynamics of IDPs and IDPRs. Mass spectrometry-based methods can provide a wide spectrum of structural characteristics, ranging from the evaluation of conformational heterogeneity of a given IDP in solution to the provision of structural description based on the rotationally averaged collision cross-sections of molecular ions, to delineation of other structural features of a protein molecule, to identification of oligomer distributions, etc.[91]

In line with this aforementioned review, Pagel et al. provided a detailed ion mobility mass spectrometry (IM-MS)-based structural description of the intrinsically disordered tumor suppressor protein p53 and a series of constructs, where the specific IDPRs (flexible linker, N and C termini) were systematically introduced to the to the 2-folded domains of this protein.[92] IM-MS is one of the mass spectrometry techniques that are frequently used to study the topology of proteins and their complexes.[93] This study revealed that p53 and constructs comprising of its ordered domains and disordered regions are collapsed in the gas phase.[92] The authors concluded that the majority of the rearrangement of p53 in the gas phase occurs

at the flexible linker and the disordered termini, which behave as unfolded protein chains that collapse in the gas phase.[92]

Rey et al. proposed to modify hydrogen/deuterium exchange mass spectrometry (HDX-MS) approach by substituting pepsin, which was almost exclusively used in these experiments for fast digestion of target proteins at pH 2–3 to retain deuterium label, by nepenthesin from the secretions of the pitcher plant Nepenthes.[94] The authors showed that nepenthesin is at least 1,400-fold more efficient than pepsin under HDX-competent conditions. Furthermore, a selectivity profile of nepenthesin is partially similar to that of pepsin, but also includes efficient cleavage C-terminal to "forbidden" residues K, R, H, and P, which promotes higher coverage of disordered regions. Therefore, nepenthesin represents a logical and highly efficient alternative to pepsin in all HDX-MS applications for the IDP/IDPR analysis.[94]

Saikusa et al. introduced a novel method for structural characterization of IDPs, IDPRs, and their complexes based on the combination of electrospray ionization ion mobility mass spectrometry (ESI-IM-MS) and SAXS.[95] Here, ESI-IM-MS is used to derive experimental collision cross-section (CCS) values, whereas SAXS serves as a source of theoretical CCS values evaluated based on the SAXS low-resolution model. The authors applied this approach for the characterization of the *Schizosaccharomyces pombe* Swi5-Sfr1 complex in which the N-terminal portion of Sfr1 is a long IDPR.[95]

Single-molecule spectroscopy. Schuler and Hofmann provided a comprehensive overview of single-molecule spectroscopy as an important method for probing protein structure and dynamics in structurally heterogeneous systems, such as IDPs.[96] It is emphasized that single-molecule Förster resonance energy transfer (FRET) and photo-induced electron transfer (PET) are uniquely positioned to investigate a wide span of timescales, and therefore provide unprecedented information on the dynamic behavior of one molecule at a time. The single-molecule spectroscopy-based techniques are used for the description of the structure and dynamics of unfolded and intrinsically disordered proteins and for the analysis of coupled folding and binding events.

Rahman et al. used single-molecule force spectroscopy, compression studies and ellipsometry to investigate the interaction modes of the intrinsically disordered acidic dehydrin TsDHN-1 and the basic dehydrin TsDHN-2 derived from the crucifer *Thellungiella salsuginea* with membranes.[97] These analyses revealed that dehydrins can stabilize lipid monolayers, induce an increase in monolayer thickness, and promote temperature-dependent phase transitions and domain formation in the supported lipid bilayers, suggesting that dehydrins interact with and potentially stabilize plant outer mitochondrial membranes in conditions of cold stress. Furthermore, single-molecule force spectroscopy analysis revealed that that binding of the dehydrins to membranes induced formation of tertiary conformations in both proteins.[97]

An application of solid-state nanopores for the single-molecule analysis of IDPs was introduced in a research paper by Japrung et al.[98] and the various aspects of generation and use of solid-state nanopores was covered in a tutorial review by the same group.[99] Here, single-molecule experiments were performed by translocating IDPs through a nanopore embedded within a thin dielectric membrane. The advantage of the approach is in the fact that the single-molecule statistics can be generated without the need of fluorescent labels or other modification groups.[98] Application of this methodology to two IDPs, a binding domain from activator of thyroid hormone and retinoid receptors (ACTR) and the nuclear coactivator binding domain of CREB-binding protein (NCBD), and to their bimolecular complex suggested conformational heterogeneity of intrinsically disordered ACTR and NCBD within the nanopore, whereas the folded ACTR-NCBD complex exhibited only one conformation when translocating through the nanopore.[98]

In cell analysis of IDPs. Atkinson et al. argued that local geometry constrains the orientational organization of the intrinsically disordered phenylalanine-glycine (FG) domains of nucleoporins (nups).[100] This conclusion was based on the use of the polarized fluorescence microscopy to characterize behavior of the FG nucleoporins in vivo. Here, the fluorescent analysis of nucleoporins tagged with green fluorescent protein along their FG domains revealed that some of these proteins were ordered, suggesting the existence of some orientational organization within the nuclear pore complex (NPC).[100]

Miscellaneous. Rogers et al. applied stopped-flow techniques to systematically analyze coupled folding and binding process in a model system where the intrinsically disordered 'BH3 region' of PUMA forms a single, contiguous α-helix upon binding the folded protein Mcl-1.[101] Comparison of the association rate constant (k^+) under a variety of solvent conditions and temperatures clearly showed that although binding was fast, the analyzed system was not 'diffusion-limited'. The authors also made a very important conclusion that standard experimental test developed for the analysis of protein-protein interactions between ordered proteins fail to provide an appropriate description of the folding/binding reactions where one protein is disordered.[101]

Proteomics of IDPs

As the number of IDPs and IDPRs in various proteomes is very large (e.g., for mammals, ~75% of their signaling proteins are predicted to contain long disordered regions (> 30 residues), about half of their total proteins are predicted to contain such long disordered regions, and ~25% of their proteins are predicted to be fully disordered), and because IDPs and IDRs have amazing structural variability and possess a very wide variety of functions, the unfoldome and unfoldomics concepts were introduced.[102-104] Papers below cover different aspects of the large scale analysis (both computational and experimental) of IDPs and IDPRs.

Oldfield et al. provided a detailed analysis of the effects of intrinsic disorder on the structure determination process and the usefulness of disorder prediction in selecting and improving proteins for structural characterization.[105] This analysis revealed that although intrinsic disorder is tolerated to some extent in crystal structures, long IDPRs are rather infrequent in solved structures. The authors also concluded that intrinsic disorder predictions represent a useful approach that

helps increasing the proportion of ordered targets in the structure determination pipeline. Surprisingly, increased success in purification was shown to be correlated with a higher proportion of predicted disordered residues in a target protein.[105] Therefore, prediction of intrinsic disorder represents an effective tool for tailoring proteins for structure determination.[105]

Tyanova et al. took a focused look at the correlation between the dynamic properties of phosphoproteome and protein structural features.[106] To this end, they studied how the variation of the amount of phosphorylation correlates with the protein structure in the vicinity of the modified site. The new twist in this study was that the analysis was done not in a static, non-quantitative way, but at six time points of the cell division cycle. The authors showed that variability of phosphorylation at a given site generally followed the degree of disorderedness in the vicinity of the modified site, with IDPRs containing dynamically varying phosphorylation levels, and with ordered regions predominantly keeping more constant phosphorylation levels.[106] This study suggested that the dynamics of phosphorylation is defined by the structural organization of the region in which a phosphorylation site resides.[106]

Sun et al. provided a comprehensive overview of various functional aspects pertaining to plant IDPs.[107] The authors emphasize the importance of IDPs in plant biology and illustrate this point by providing description of the multifarious roles of intrinsic disorder in five different types of plant protein families experimentally confirmed as IDPs. Functions ascribed to plant IDPs possess broad impact on many areas of plant biology, such as transcriptional regulation, light perception, abiotic stress, and plant development.[107]

In agreement with this work,[107] study by Pietrosemoli et al. revealed that IDPs and proteins with IDPRs are rather common in *Arabidopsis thaliana*, the most widely used model organism in plant biology.[108] This analysis also revealed that many Arabidopsis proteins related to environmental response were significantly enriched in disorder. Based on these observations the authors proposed that the increased level of intrinsic disorder in the environmental response-related proteins in plants represents a useful defense mechanism that allows plants to quickly adapt and respond to challenging environmental conditions.[108]

Also in line with this work,[107] Wood et al. showed that Actinidia DRM1 (Ade DORMANCY ASSOCIATED GENE 1), a plant-specific IDP, represents a robust dormancy marker whose mRNA transcript expression exhibits a strong inverse correlation with the onset of growth following periods of physiological dormancy.[109] The intrinsically disordered nature of this protein was suggested by several bioinformatics tools, and several DRM1 homologs, which align into two distinct Actinidia-specific families, Type I and Type II, have been identified.[109]

Costantini et al. investigated the distribution of the structural features of N- and C-terminal segments of sirtuins in all known organisms to understand their evolutionary histories by taking into account average length of terminal segments, amino acid composition, intrinsic disorder, presence of charged stretches, presence of putative phosphorylation sites, flexibility, and GC content of genes.[110] This study revealed that this ancient family of proteins contain multiple functionally crucial IDPRs.[110]

Coelho Ribeiro et al. used a wide array of bioinformatics methods to analyze the abundance and functional roles of intrinsic disorder in proteins involved in the formation of the yeast spliceosome.[111] The spliceosome is a multimegadalton ribonucleoprotein machine catalyzing the excision of introns from eukaryotic pre-mRNAs, which, in yeast, consists of five small nuclear RNAs (U1, U2, U4, U5, and U6) and a range of associated proteins. The analysis revealed that intrinsic disorder is abundant in all spliceosome-related proteins, being found both, in proteins constituting cores of the ribonucleoproteins (snRNPs) and in numerous auxiliary proteins.[111]

Looking at IDPs/IDPRs in Diseases

Because IDPs play crucial roles in numerous biological processes, being involved in control and regulation of almost all crucial processes inside the cell, many of these proteins are implicated in human disease.[112,113] This conclusion is based on analysis of the pathological roles of several individual IDPs and extensive computational/bioinformatics studies addressing the abundance of IDPs in various pathological conditions, including cancer and neurodegenerative diseases.[15,30,112-124]

IDPs in cancer. Mahmoudabadi et al. created and analyzed a model of a protein network with the topological characteristics of a cancer protein network.[125] The cornerstone idea of this model are the protein intrinsic disorder-based concepts of conformational noise and transcriptional (genetic) noise that define activation of hidden and dormant pathways some of which are responsible for cellular transformation in cancer and other state-switching events. Conformational noise characterizes the stochastic interactions between IDPs and their partners and defines an ensemble of protein network configurations, from which the most suitable can be explored in response to perturbations, conferring protein networks with remarkable flexibility and resilience.[125] Transcriptional noise on the other hand is defined by the disorderedness of transcription factors and hub proteins. Therefore, IDPs serve as master controllers and effectors of transcriptional and conformational noise, playing crucial roles in rewiring protein networks and unmasking dormant interactions in response to perturbations.[125]

Analysis of mixed lineage leukemia (MLL) fusion proteins by Leach et al. revealed that these IDPs play a number of important roles in normal hematopoiesis and in acute leukemia.[126] The authors studied the ANC1 homology domain (AHD) of one of the most common MLL fusion proteins, AF9, that promotes oncogenic transformation of hematopoietic cells by constitutive recruitment of elongation factors to HOX promoters, resulting in overexpression of target genes. AF9 AHD was shown to undergo coupled folding and binding as a result of interaction with multiple transcription factors.[126] Importantly, AF9 AHD is engaged in the formation of fuzzy complexes, were it retains significant dynamic behavior in the bound form which may facilitate exchange between disordered partners.[126]

Wang et al. provided a comprehensive overview of the current state of the art in the field of the structural and functional characterization of highly conserved coactivators CREB-binding protein (CBP) and its paralog, E1A-binding protein (p300).[127] CBP/p300 is one of the important coactivators that promote transcription by connecting transcription factors to the basal transcriptional machinery. This protein has several functional domains (e.g., four separate transactivation domains (TADs) that interact with the TADs of a number of DNA-binding transcription activators and general transcription factors, a catalytic histone acetyltransferase (HAT) domain and several IDPRs) and is involved in multivalent interactions with the various components of the transcription machinery. Because of the multitude of its transcriptional and epigenetic functions, CBP/p300 is involved in a set of complex physiological and pathological processes (such as proliferation or apoptosis), and dysregulation of this protein is associated with leukemia and other types of cancer.[127]

IDPs in neurodegenerative diseases. Work of Ariesandi et al. unraveled the role of pre-existing oligomeric species in promoting α-synuclein fibrillation.[128] These authors established that the heat pretreatment depleted the amount of rare pre-existing α-synuclein oligomers leading to the dramatic inhibition of this protein fibrillation under the ambient temperatures.[128]

Singh et al. showed that the polyphenolic ingredient of Asian food, curcumin, reduces toxicity of α-synuclein aggregates by binding to preformed oligomers and fibrils and modifying the exposure degree of their hydrophobic surfaces.[129] Fluorescence and 2D-NMR analysis revealed that there is a specific binding of curcumin to the oligomeric intermediates, whereas monomeric protein does not interact with this polyphenol. Importantly, although the addition of curcumin resulted in the accelerated aggregation α-synuclein, the population of toxic oligomeric intermediates of this protein was noticeably reduced.[129]

Maltsev et al. investigated site-specific interaction between α-synuclein and membranes using the analysis of the NMR-detected methionine oxidation rates.[130] In this work, the ability of membrane containing a small fraction of peroxidized lipids to rapidly oxidize the N-terminal methionine residues in α-synuclein (Met1 and Met5) was utilized. This study showed that the oxidation rates of Met1 and Met5 were mutually reduces as a result of decreased membrane affinity of the partially oxidized protein.[130]

Among three point mutations of α-synuclein associated with the familial cases of the early onset Parkinson disease, the E46K genetic missense mutation is the most recent addition. Wise-Scira et al. analyzed the effect of this mutation (which was originally identified in a family of Spanish origin with hereditary Parkinson disease) on the structural properties, conformations, and free energy landscape of α-synuclein using the extensive parallel tempering molecular dynamics simulations along with thermodynamic calculations.[131] The authors also used the recently developed theoretical strategy for calculation of the free energy change values associated with the transitions between two different secondary structure components for each residue of the wild-type and E46K mutant of α-synuclein. This study revealed that in addition to obvious local changes, the E46K mutation induced noticeable long-range alterations in the structural properties of α-synuclein. In particular, E46K mutation promoted a significant decrease in helical content accompanied by a large increase in β-sheet structure propensity.[131] Furthermore, E46K mutation was shown to increase the existing long-range intramolecular interactions between the C-terminal region and the N-terminal and NAC regions of α-synuclein, leading to the formation of more compact structures.[131]

In another study by the same group, the effects of another familial mutation (A30P) on structure, dynamic properties, and free energy landscapes of this interesting protein were investigated by the analogous theoretical approach.[132] This analysis showed that helical content of Ala18-Gly31 region of the mutant α-synuclein was less prominent in comparison to the wild-type protein. Also, mutation affected the long-range intramolecular protein interactions making them less abundant, especially between the NAC and C-terminal regions, leading to the less compact and less stable structures of the A30P mutant.[132]

Rabe et al. reported that α-synuclein can spontaneously aggregate at concentrations as low as 1 nM and below (i.e., far lower than physiological concentrations of this protein) in the presence of both hydrophilic glass surfaces and cell membrane mimicking supported lipid bilayers.[133] The authors used three-dimensional supercritical angle fluorescence (3D-SAF) microscopy to follow the α-synuclein aggregation process in situ. This analysis revealed the heterogeneous nature of the aggregation process, where two different types of α-synuclein aggregates were formed. The first type was completely adsorbed to the surface and grew along the surface plane, whereas the second type of extended was tethered with one end to the surface being mobile at the other end. The growing mechanisms of these two amyloid-like structures were significantly different.[133]

Gurry et al. investigated the aggregation behavior of an α-synuclein construct containing a 10-residue N-terminal extension.[134] Data from NMR chemical shifts and residual dipolar couplings were used to generate the conformational ensemble of this construct. Analysis of this conformational ensemble showed that a disordered monomer was the dominant state of this ensemble, complemented by a small fraction of α-helical and β-structural trimers and tetramers.[134]

Fantini et al. delved into the analysis of the molecular mechanisms underlying the ganglioside- and cholesterol-controlled interaction of α-synuclein with neural membranes.[135] The mechanistic model is proposed where α-synuclein first interacts with a cell surface glycosphingolipid. This primary binding to ganglioside GM3 in astrocytes or GM1 in neurons induces partial folding of an α-helical domain that contains a high affinity binding motif (67–78 peptide) for cholesterol. At the last stage of the insertion process, an oligomeric channel is formed.[135]

Viral IDPs. Schulze-Gahmen et al. investigated the molecular mechanisms of the selective recruitment of the human

positive transcription elongation factor b (P-TEFb) that phosphorylates RNA polymerase II and regulatory proteins to trigger elongation of many gene transcripts by the HIV-1 Tat protein.[136] The authors solved the structure a tripartite super elongation complex (SEC) containing P-TEFb, flexible AFF4 scaffold and HIV Tat. This study revealed that AFF4 is involved in the direct interaction with HIV Tat and that Tat enhanced P-TEFb affinity for AFF4.[136]

IDPs/IDPRs as Drugs or Drug Targets

Since many proteins associated with various human diseases are either completely disordered or contain long disordered regions,[112,113] and since some of these disease-related IDPs/IDPRs are involved in recognition, regulation and signaling, these proteins/regions clearly represent novel potential drug targets.[137] There are at least two potential approaches for the inhibition of the disorder-based interactions, where small molecule either bind to the binding site of the ordered partner to outcompete the IDPs/IDPRs or interacts directly with the IDP/IDPR. Importantly, small molecules can inhibit disorder-based protein-protein interactions via induction of the dysfunctional ordered structures in targeted IDPR; i.e., via the drug-induced misfolding.[37,137]

Rawat et al. used NMR to investigate the backbone dynamics and conformational properties of drug peptide salmon calcitonin (sCT) in aqueous solution.[138] This analysis revealed that sCT being mostly unfolded in solution, contained noticeable residual structure organized in multiple conformational states exchanging slowly on the NMR timescale (in a range of 10^2–10^3 s^{-1}). More detailed analysis suggested that sCT is structurally heterogeneous and contains highly flexible C-terminal region (residues Thr25-Thr31) that is needed for the identification of specific target receptors, and more structured N-terminal region (residues Cys1-Gln20) possessing significant amount of conformational plasticity and strong bias toward biologically active α-helical structure that is responsible for favorable functional adaptation.[138]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest disclosed.

References

1. Uversky VN, Dunker AK. Multiparametric analysis of intrinsically disordered proteins: looking at intrinsic disorder through compound eyes. Anal Chem 2012; 84:2096-104; PMID:22242801; http://dx.doi.org/10.1021/ac203096k.
2. Uversky VN, Dunker AK. Understanding protein nonfolding. Biochim Biophys Acta 2010; 1804:1231-64; PMID:20117254; http://dx.doi.org/10.1016/j.bbapap.2010.01.017.
3. Dunker AK, Obradovic Z. The protein trinity--linking function and disorder. Nat Biotechnol 2001; 19:805-6; PMID:11533628; http://dx.doi.org/10.1038/nbt0901-805.
4. Dunker AK, Lawson JD, Brown CJ, Williams RM, Romero P, Oh JS, et al. Intrinsically disordered protein. J Mol Graph Model 2001; 19:26-59; PMID:11381529; http://dx.doi.org/10.1016/S1093-3263(00)00138-8.
5. Uversky VN. Natively unfolded proteins: a point where biology waits for physics. Protein Sci 2002; 11:739-56; PMID:11910019; http://dx.doi.org/10.1110/ps.4210102.
6. Uversky VN. What does it mean to be natively unfolded? Eur J Biochem 2002; 269:2-12; PMID:11784292; http://dx.doi.org/10.1046/j.0014-2956.2001.02649.x.
7. Daughdrill GW, Pielak GJ, Uversky VN, Cortese MS, Dunker AK. Natively disordered proteins. In: Buchner J, Kiefhaber T, eds. Handbook of Protein Folding. Weinheim, Germany: Wiley-VCH, Verlag GmbH & Co. KGaA, 2005:271-353.
8. Uversky VN. Unusual biophysics of intrinsically disordered proteins. Biochim Biophys Acta 2013; 1834:932-51; PMID:23269364; http://dx.doi.org/10.1016/j.bbapap.2012.12.008.
9. Uversky VN. A decade and a half of protein intrinsic disorder: Biology still waits for physics. Protein Sci 2013; 22:693-724; PMID:23553817; http://dx.doi.org/10.1002/pro.2261.
10. Mao AH, Lyle N, Pappu RV. Describing sequence-ensemble relationships for intrinsically disordered proteins. Biochem J 2013; 449:307-18; PMID:23240611; http://dx.doi.org/10.1042/BJ20121346.
11. Tantos A, Szrnka K, Szabo B, Bokor M, Kamasa P, Matus P, et al. Structural disorder and local order of hNopp140. Biochim Biophys Acta 2013; 1834:342-50; PMID:22906532; http://dx.doi.org/10.1016/j.bbapap.2012.08.005.
12. Marasini C, Galeno L, Moran O. A SAXS-based ensemble model of the native and phosphorylated regulatory domain of the CFTR. Cell Mol Life Sci 2013; 70:923-33; PMID:23052212; http://dx.doi.org/10.1007/s00018-012-1172-5.
13. Elam WA, Schrank TP, Campagnolo AJ, Hilser VJ. Temperature and urea have opposing impacts on polyproline II conformational bias. Biochemistry 2013; 52:949-58; PMID:23350874; http://dx.doi.org/10.1021/bi301435p.
14. Mizuguchi M, Okazawa H. Structural study of polyglutamine tract-binding protein 1. Yakugaku Zasshi 2013; 133:519-26; PMID:23649393; http://dx.doi.org/10.1248/yakushi.13-00001-2.
15. Iakoucheva LM, Brown CJ, Lawson JD, Obradovic Z, Dunker AK. Intrinsic disorder in cell-signaling and cancer-associated proteins. J Mol Biol 2002; 323:573-84; PMID:12381310; http://dx.doi.org/10.1016/S0022-2836(02)00969-5.
16. Dunker AK, Cortese MS, Romero P, Iakoucheva LM, Uversky VN. Flexible nets. The roles of intrinsic disorder in protein interaction networks. FEBS J 2005; 272:5129-48; PMID:16218947; http://dx.doi.org/10.1111/j.1742-4658.2005.04948.x.
17. Uversky VN, Oldfield CJ, Dunker AK. Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. J Mol Recognit 2005; 18:343-84; PMID:16094605; http://dx.doi.org/10.1002/jmr.747.
18. Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM, Obradovic Z. Intrinsic disorder and protein function. Biochemistry 2002; 41:6573-82; PMID:12022860; http://dx.doi.org/10.1021/bi012159+.
19. Iakoucheva LM, Radivojac P, Brown CJ, O'Connor TR, Sikes JG, Obradovic Z, et al. The importance of intrinsic disorder for protein phosphorylation. Nucleic Acids Res 2004; 32:1037-49; PMID:14960716; http://dx.doi.org/10.1093/nar/gkh253.
20. Dyson HJ, Wright PE. Coupling of folding and binding for unstructured proteins. Curr Opin Struct Biol 2002; 12:54-60; PMID:11839490; http://dx.doi.org/10.1016/S0959-440X(02)00289-0.
21. Oldfield CJ, Cheng Y, Cortese MS, Romero P, Uversky VN, Dunker AK. Coupled folding and binding with alpha-helix-forming molecular recognition elements. Biochemistry 2005; 44:12454-70; PMID:16156658; http://dx.doi.org/10.1021/bi050736e.
22. Oldfield CJ, Meng J, Yang JY, Yang MQ, Uversky VN, Dunker AK. Flexible nets: disorder and induced fit in the associations of p53 and 14-3-3 with their partners. BMC Genomics 2008; 9(Suppl 1):S1; PMID:18366598; http://dx.doi.org/10.1186/1471-2164-9-S1-S1.
23. Hsu WL, Oldfield CJ, Xue B, Meng J, Huang F, Romero P, et al. Exploring the binding diversity of intrinsically disordered proteins involved in one-to-many binding. Protein Sci 2013; 22:258-73; PMID:23233352; http://dx.doi.org/10.1002/pro.2207.
24. Fuxreiter M, Tompa P. Fuzzy complexes: a more stochastic view of protein function. Adv Exp Med Biol 2012; 725:1-14; PMID:22399315; http://dx.doi.org/10.1007/978-1-4614-0659-4_1.
25. Tompa P, Fuxreiter M. Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions. Trends Biochem Sci 2008; 33:2-8; PMID:18054235; http://dx.doi.org/10.1016/j.tibs.2007.10.003.
26. Uversky VN. Multitude of binding modes attainable by intrinsically disordered proteins: a portrait gallery of disorder-based complexes. Chem Soc Rev 2011; 40:1623-34; PMID:21049125; http://dx.doi.org/10.1039/c0cs00057d.
27. Gsponer J, Futschik ME, Teichmann SA, Babu MM. Tight regulation of unstructured proteins: from transcript synthesis to protein degradation. Science 2008; 322:1365-8; PMID:19039133; http://dx.doi.org/10.1126/science.1163581.
28. Uversky VN, Dunker AK. Biochemistry. Controlled chaos. Science 2008; 322:1340-1; PMID:19039128; http://dx.doi.org/10.1126/science.1167453.
29. Tompa P. The interplay between structure and function in intrinsically unstructured proteins. FEBS Lett 2005; 579:3346-54; PMID:15943980; http://dx.doi.org/10.1016/j.febslet.2005.03.072.
30. Xie H, Vucetic S, Iakoucheva LM, Oldfield CJ, Dunker AK, Uversky VN, et al. Functional anthology of intrinsic disorder. 1. Biological processes and functions of proteins with long disordered regions. J Proteome Res 2007; 6:1882-98; PMID:17391014; http://dx.doi.org/10.1021/pr060392u.
31. Grimmler M, Wang Y, Mund T, Cilensek Z, Keidel EM, Waddell MB, et al. Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. Cell 2007; 128:269-80; PMID:17254966; http://dx.doi.org/10.1016/j.cell.2006.11.047.
32. Uversky VN, Dunker AK. The case for intrinsically disordered proteins playing contributory roles in molecular recognition without a stable 3D structure. F1000 Biol Rep 2013; 5:1; PMID:23361308; http://dx.doi.org/10.3410/B5-01.

33. Janin J, Sternberg MJ. Protein flexibility, not disorder, is intrinsic to molecular recognition. F1000 Biol Rep 2013; 5:2; PMID:23361309; http://dx.doi.org/10.3410/B5-02.
34. Landré V, Pion E, Narayan V, Xirodimas DP, Ball KL. DNA-binding regulates site-specific ubiquitination of IRF-1. Biochem J 2013; 449:707-17; PMID:23134341; http://dx.doi.org/10.1042/BJ20121076.
35. Das RK, Mittal A, Pappu RV. How is functional specificity achieved through disordered regions of proteins? Bioessays 2013; 35:17-22; PMID:23138868; http://dx.doi.org/10.1002/bies.201200115.
36. Kovacs D, Szabo B, Pancsa R, Tompa P. Intrinsically disordered proteins undergo and assist folding transitions in the proteome. Arch Biochem Biophys 2013; 531:80-9; PMID:23142500; http://dx.doi.org/10.1016/j.abb.2012.09.010.
37. Uversky VN. Intrinsic Disorder-based Protein Interactions and their Modulators. Curr Pharm Des 2013; 19:4191-213; PMID:23170892; http://dx.doi.org/10.2174/1381612811319230005.
38. Barbour KW, Xing YY, Peña EA, Berger FG. Characterization of the bipartite degron that regulates ubiquitin-independent degradation of thymidylate synthase. Biosci Rep 2013; 33:165-73; PMID:23181752; http://dx.doi.org/10.1042/BSR20120112.
39. Dubé MP, Castonguay Y, Cloutier J, Michaud J, Bertrand A. Characterization of two novel cold-inducible K3 dehydrin genes from alfalfa (Medicago sativa spp. sativa L.). Theor Appl Genet 2013; 126:823-35; PMID:23188214; http://dx.doi.org/10.1007/s00122-012-2020-6.
40. Wang X, Xiong LW, El Ayadi A, Boehning D, Putkey JA. The calmodulin regulator protein, PEP-19, sensitizes ATP-induced Ca2+ release. J Biol Chem 2013; 288:2040-8; PMID:23204517; http://dx.doi.org/10.1074/jbc.M112.411314.
41. Banci L, Bertini I, Cefaro C, Ciofi-Baffoni S, Gajda K, Felli IC, et al. An intrinsically disordered domain has a dual function coupled to compartment-dependent redox control. J Mol Biol 2013; 425:594-608; PMID:23207295; http://dx.doi.org/10.1016/j.jmb.2012.11.032.
42. Bansal N, Zhang M, Bhaskar A, Itotia P, Lee E, Shlyakhtenko LS, et al. Assembly of the SLIP1-SLBP complex on histone mRNA requires heterodimerization and sequential binding of SLBP followed by SLIP1. Biochemistry 2013; 52:520-36; PMID:23286197; http://dx.doi.org/10.1021/bi301074r.
43. Drobnak I, De Jonge N, Haesaerts S, Vesnaver G, Loris R, Lah J. Energetic basis of uncoupling folding from binding for an intrinsically disordered protein. J Am Chem Soc 2013; 135:1288-94; PMID:23289531; http://dx.doi.org/10.1021/ja305081b.
44. Khan H, Cino EA, Brickenden A, Fan J, Yang D, Choy WY. Fuzzy complex formation between the intrinsically disordered prothymosin α and the Kelch domain of Keap1 involved in the oxidative stress response. J Mol Biol 2013; 425:1011-27; PMID:23318954; http://dx.doi.org/10.1016/j.jmb.2013.01.005.
45. Huang KL, Chadee AB, Chen CY, Zhang Y, Shyu AB. Phosphorylation at intrinsically disordered regions of PAM2 motif-containing proteins modulates their interactions with PABPC1 and influences mRNA fate. RNA 2013; 19:295-305; PMID:23340509; http://dx.doi.org/10.1261/rna.037317.112.
46. Chen L, Brown JW, Mok YF, Hatters DM, McKnight CJ. The allosteric mechanism induced by protein kinase A (PKA) phosphorylation of dematin (band 4.9). J Biol Chem 2013; 288:8313-20; PMID:23355471; http://dx.doi.org/10.1074/jbc.M112.438861.
47. Kjaergaard M, Andersen L, Nielsen LD, Teilum K. A Folded Excited State of Ligand-Free Nuclear Coactivator Binding Domain (NCBD) Underlies Plasticity in Ligand Recognition. Biochemistry 2013.
48. Hayward DC, Dosztányi Z, Clark-Walker GD. The N-terminal intrinsically disordered domain of Mgm101p is localized to the mitochondrial nucleoid. PLoS One 2013; 8:e56465; PMID:23418572; http://dx.doi.org/10.1371/journal.pone.0056465.
49. Maestro B, Galán B, Alfonso C, Rivas G, Prieto MA, Sanz JM. A new family of intrinsically disordered proteins: structural characterization of the major phasin PhaF from Pseudomonas putida KT2440. PLoS One 2013; 8:e56904; PMID:23457638; http://dx.doi.org/10.1371/journal.pone.0056904.
50. Zhang M, Pascal JM, Zhang JF. Unstructured to structured transition of an intrinsically disordered protein peptide in coupling Ca^{2+}-sensing and SK channel activation. Proc Natl Acad Sci U S A 2013; 110:4828-33; PMID:23487779; http://dx.doi.org/10.1073/pnas.1220253110.
51. Uversky VN, Gillespie JR, Fink AL. Why are "natively unfolded" proteins unstructured under physiologic conditions? Proteins 2000; 41:415-27; PMID:11025552; http://dx.doi.org/10.1002/1097-0134(20001115)41:3<415::AID-PROT130>3.0.CO;2-7.
52. Dunker AK, Garner E, Guilliot S, Romero P, Albrecht K, Hart J, et al. Protein disorder and the evolution of molecular recognition: theory, predictions and observations. Pac Symp Biocomput 1998; •••:473-84; PMID:9697205.
53. Radivojac P, Iakoucheva LM, Oldfield CJ, Obradovic Z, Uversky VN, Dunker AK. Intrinsic disorder and functional proteomics. Biophys J 2007; 92:1439-56; PMID:17158572; http://dx.doi.org/10.1529/biophysj.106.094045.
54. Vacic V, Uversky VN, Dunker AK, Lonardi S. Composition Profiler: a tool for discovery and visualization of amino acid composition differences. BMC Bioinformatics 2007; 8:211; PMID:17578581; http://dx.doi.org/10.1186/1471-2105-8-211.
55. He B, Wang K, Liu Y, Xue B, Uversky VN, Dunker AK. Predicting intrinsic disorder in proteins: an overview. Cell Res 2009; 19:929-49; PMID:19597536; http://dx.doi.org/10.1038/cr.2009.87.
56. Oldfield CJ, Cheng Y, Cortese MS, Brown CJ, Uversky VN, Dunker AK. Comparing and combining predictors of mostly disordered proteins. Biochemistry 2005; 44:1989-2000; PMID:15697224; http://dx.doi.org/10.1021/bi047993o.
57. Ferron F, Longhi S, Canard B, Karlin D. A practical overview of protein disorder prediction methods. Proteins 2006; 65:1-14; PMID:16856179; http://dx.doi.org/10.1002/prot.21075.
58. Esnouf RM, Hamer R, Sussman JL, Silman I, Trudgian D, Yang ZR, et al. Honing the in silico toolkit for detecting protein disorder. Acta Crystallogr D Biol Crystallogr 2006; 62:1260-6; PMID:17001103; http://dx.doi.org/10.1107/S0907444906033580.
59. Bourhis JM, Canard B, Longhi S. Predicting protein disorder and induced folding: from theoretical principles to practical applications. Curr Protein Pept Sci 2007; 8:135-49; PMID:17430195; http://dx.doi.org/10.2174/138920307780363451.
60. Dosztányi Z, Sándor M, Tompa P, Simon I. Prediction of protein disorder at the domain level. Curr Protein Pept Sci 2007; 8:161-71; PMID:17430197; http://dx.doi.org/10.2174/138920307780363406.
61. Dosztányi Z, Tompa P. Prediction of protein disorder. Methods Mol Biol 2008; 426:103-15; PMID:18542859; http://dx.doi.org/10.1007/978-1-60327-058-8_6.
62. Huang F, Oldfield C, Meng J, Hsu WL, Xue B, Uversky VN, et al. Subclassifying disordered proteins by the CH-CDF plot method. Pac Symp Biocomput 2012; •••:128-39; PMID:22174269.
63. Huang T, He ZS, Cui WR, Cai YD, Shi XH, Hu LL, et al. A sequence-based approach for predicting protein disordered regions. Protein Pept Lett 2013; 20:243-8; PMID:22591473.
64. Jin F, Liu Z. Inherent relationships among different biophysical prediction methods for intrinsically disordered proteins. Biophys J 2013; 104:488-95; PMID:23442871; http://dx.doi.org/10.1016/j.bpj.2012.12.012.
65. Ostermeir K, Zacharias M. Advanced replica-exchange sampling to study the flexibility and plasticity of peptides and proteins. Biochim Biophys Acta 2013; 1834:847-53; PMID:23298543; http://dx.doi.org/10.1016/j.bbapap.2012.12.016.
66. Mittal J, Yoo TH, Georgiou G, Truskett TM. Structural ensemble of an intrinsically disordered polypeptide. J Phys Chem B 2013; 117:118-24; PMID:23205890; http://dx.doi.org/10.1021/jp308984e.
67. McDowell C, Chen J, Chen J. Potential conformational heterogeneity of p53 bound to S100B(ββ). J Mol Biol 2013; 425:999-1010; PMID:23313430; http://dx.doi.org/10.1016/j.jmb.2013.01.001.
68. Krzeminski M, Marsh JA, Neale C, Choy WY, Forman-Kay JD. Characterization of disordered proteins with ENSEMBLE. Bioinformatics 2013; 29:398-9; PMID:23233655; http://dx.doi.org/10.1093/bioinformatics/bts701.
69. Matsushita K, Kikuchi M. Frustration-induced protein intrinsic disorder. J Chem Phys 2013; 138:105101; PMID:23514521; http://dx.doi.org/10.1063/1.4794781.
70. Bryngelson JD, Wolynes PG. Spin glasses and the statistical mechanics of protein folding. Proc Natl Acad Sci U S A 1987; 84:7524-8; PMID:3478708; http://dx.doi.org/10.1073/pnas.84.21.7524.
71. Leopold PE, Montal M, Onuchic JN. Protein folding funnels: a kinetic approach to the sequence-structure relationship. Proc Natl Acad Sci U S A 1992; 89:8721-5; PMID:1528885; http://dx.doi.org/10.1073/pnas.89.18.8721.
72. Receveur-Bréchot V, Bourhis JM, Uversky VN, Canard B, Longhi S. Assessing protein disorder and induced folding. Proteins 2006; 62:24-45; PMID:16287116; http://dx.doi.org/10.1002/prot.20750.
73. Eliezer D. Biophysical characterization of intrinsically disordered proteins. Curr Opin Struct Biol 2009; 19:23-30; PMID:19162471; http://dx.doi.org/10.1016/j.sbi.2008.12.004.
74. Jensen MR, Salmon L, Nodet G, Blackledge M. Defining conformational ensembles of intrinsically disordered and partially folded proteins directly from chemical shifts. J Am Chem Soc 2010; 132:1270-2; PMID:20063887; http://dx.doi.org/10.1021/ja909973n.
75. Longhi S, Uversky VN, eds. Instrumental Analysis of Intrinsically Disordered Proteins: Assessing Structure and Conformation. Hoboken, New Jersey, USA: John Wiley & Sons, Inc, 2010.
76. Uversky VN, Dunker AK, eds. Experimental Tools for the Analysis of Intrinsically Disordered Protein: Volume I Totowa, NJ USA: Humana Press, 2012.
77. Uversky VN, Dunker AK, eds. Experimental Tools for the Analysis of Intrinsically Disordered Protein: Volume II Totowa, NJ USA: Humana Press, 2012.
78. Ota M, Koike R, Amemiya T, Tenno T, Romero PR, Hiroaki H, et al. An assignment of intrinsically disordered regions of proteins based on NMR structures. J Struct Biol 2013; 181:29-36; PMID:23142703; http://dx.doi.org/10.1016/j.jsb.2012.10.017.
79. Nyarko A, Song Y, Novácek J, Žídek L, Barbar E. Multiple recognition motifs in nucleoporin Nup159 provide a stable and rigid Nup159-Dyn2 assembly. J Biol Chem 2013; 288:2614-22; PMID:23223634; http://dx.doi.org/10.1074/jbc.M112.432831.
80. Yamaguchi T, Uno T, Uekusa Y, Yagi-Utsumi M, Kato K. Ganglioside-embedding small bicelles for probing membrane-landing processes of intrinsically disordered proteins. Chem Commun (Camb) 2013; 49:1235-7; PMID:23296043; http://dx.doi.org/10.1039/c2cc38016a.

81. Prestegard JH, Sahu SC, Nkari WK, Morris LC, Live D, Gruta C. Chemical shift prediction for denatured proteins. J Biomol NMR 2013; 55:201-9; PMID:23297019; http://dx.doi.org/10.1007/s10858-012-9702-x.
82. Bermel W, Bruix M, Felli IC, Kumar M V V, Pierattelli R, Serrano S. Improving the chemical shift dispersion of multidimensional NMR spectra of intrinsically disordered proteins. J Biomol NMR 2013; 55:231-7; PMID:23314728; http://dx.doi.org/10.1007/s10858-013-9704-3.
83. Kim S, Wu KP, Baum J. Fast hydrogen exchange affects ^{15}N relaxation measurements in intrinsically disordered proteins. J Biomol NMR 2013; 55:249-56; PMID:23314729; http://dx.doi.org/10.1007/s10858-013-9706-1.
84. Pham ND, Parker RB, Kohler JJ. Photocrosslinking approaches to interactome mapping. Curr Opin Chem Biol 2013; 17:90-101; PMID:23149092; http://dx.doi.org/10.1016/j.cbpa.2012.10.034.
85. Neira JL. Nuclear magnetic resonance spectroscopy to study virus structure. Subcell Biochem 2013; 68:145-76; PMID:23737051; http://dx.doi.org/10.1007/978-94-007-6552-8_5.
86. Martinho M, Habchi J, El Habre Z, Nesme L, Guigliarelli B, Belle V, et al. Assessing induced folding within the intrinsically disordered C-terminal domain of the Henipavirus nucleoproteins by site-directed spin labeling EPR spectroscopy. J Biomol Struct Dyn 2013; 31:453-71; PMID:22881220; http://dx.doi.org/10.1080/07391102.2012.706068.
87. Longhi S, Belle V, Fournel A, Guigliarelli B, Carrière F. Probing structural transitions in both structured and disordered proteins using site-directed spin-labeling EPR spectroscopy. J Pept Sci 2011; 17:315-28; PMID:21351321; http://dx.doi.org/10.1002/psc.1344.
88. Drescher M. EPR in protein science: intrinsically disordered proteins. Top Curr Chem 2012; 321:91-119; PMID:21826602; http://dx.doi.org/10.1007/128_2011_235.
89. Somkuti J, Mártonfalvi Z, Kellermayer MS, Smeller L. Different pressure-temperature behavior of the structured and unstructured regions of titin. Biochim Biophys Acta 2013; 1834:112-8; PMID:23063534; http://dx.doi.org/10.1016/j.bbapap.2012.10.001.
90. Cortese MS, Uversky VN, Dunker AK. Intrinsic disorder in scaffold proteins: getting more from less. Prog Biophys Mol Biol 2008; 98:85-106; PMID:18619997; http://dx.doi.org/10.1016/j.pbiomolbio.2008.05.007.
91. Beveridge R, Chappuis Q, Macphee C, Barran P. Mass spectrometry methods for intrinsically disordered proteins. Analyst 2013; 138:32-42; PMID:23108160; http://dx.doi.org/10.1039/c2an35665a.
92. Pagel K, Natan E, Hall Z, Fersht AR, Robinson CV. Intrinsically disordered p53 and its complexes populate compact conformations in the gas phase. Angew Chem Int Ed Engl 2013; 52:361-5; PMID:22777995; http://dx.doi.org/10.1002/anie.201203047.
93. Bohrer BC, Merenbloom SI, Koeniger SL, Hilderbrand AE, Clemmer DE. Biomolecule analysis by ion mobility spectrometry. Annu Rev Anal Chem (Palo Alto Calif) 2008; 1:293-327; PMID:20636082; http://dx.doi.org/10.1146/annurev.anchem.1.031207.113001.
94. Rey M, Yang M, Burns KM, Yu Y, Lees-Miller SP, Schriemer DC. Nepenthesin from monkey cups for hydrogen/deuterium exchange mass spectrometry. Mol Cell Proteomics 2013; 12:464-72; PMID:23197791; http://dx.doi.org/10.1074/mcp.M112.025221.
95. Saikusa K, Kuwabara N, Kokabu Y, Inoue Y, Sato M, Iwasaki H, et al. Characterisation of an intrinsically disordered protein complex of Swi5-Sfr1 by ion mobility mass spectrometry and small-angle X-ray scattering. Analyst 2013; 138:1441-9; PMID:23324799; http://dx.doi.org/10.1039/c2an35878f.
96. Schuler B, Hofmann H. Single-molecule spectroscopy of protein folding dynamics--expanding scope and timescales. Curr Opin Struct Biol 2013; 23:36-47; PMID:23312353; http://dx.doi.org/10.1016/j.sbi.2012.10.008.
97. Rahman LN, McKay F, Giuliani M, Quirk A, Moffatt BA, Harauz G, et al. Interactions of Thellungiella salsuginea dehydrins TsDHN-1 and TsDHN-2 with membranes at cold and ambient temperatures-surface morphology and single-molecule force measurements show phase separation, and reveal tertiary and quaternary associations. Biochim Biophys Acta 2013; 1828:967-80; PMID:23219803; http://dx.doi.org/10.1016/j.bbamem.2012.11.031.
98. Japrung D, Dogan J, Freedman KJ, Nadzeyka A, Bauerdick S, Albrecht T, et al. Single-molecule studies of intrinsically disordered proteins using solid-state nanopores. Anal Chem 2013; 85:2449-56; PMID:23327569; http://dx.doi.org/10.1021/ac3035025.
99. Miles BN, Ivanov AP, Wilson KA, Dogan F, Japrung D, Edel JB. Single molecule sensing with solid-state nanopores: novel materials, methods, and applications. Chem Soc Rev 2013; 42:15-28; PMID:22990878; http://dx.doi.org/10.1039/c2cs35286a.
100. Atkinson CE, Mattheyses AL, Kampmann M, Simon SM. Conserved spatial organization of FG domains in the nuclear pore complex. Biophys J 2013; 104:37-50; PMID:23332057; http://dx.doi.org/10.1016/j.bpj.2012.11.3823.
101. Rogers JM, Steward A, Clarke J. Folding and binding of an intrinsically disordered protein: fast, but not 'diffusion-limited'. J Am Chem Soc 2013; 135:1415-22; PMID:23301700; http://dx.doi.org/10.1021/ja309527h.
102. Cortese MS, Baird JP, Uversky VN, Dunker AK. Uncovering the unfoldome: enriching cell extracts for unstructured proteins by acid treatment. J Proteome Res 2005; 4:1610-8; PMID:16212413; http://dx.doi.org/10.1021/pr050119c.
103. Dunker AK, Oldfield CJ, Meng J, Romero P, Yang JY, Chen JW, et al. The unfoldomics decade: an update on intrinsically disordered proteins. BMC Genomics 2008; 9(Suppl 2):S1; PMID:18831774; http://dx.doi.org/10.1186/1471-2164-9-S2-S1.
104. Midic U, Oldfield CJ, Dunker AK, Obradovic Z, Uversky VN. Protein disorder in the human diseasome: unfoldomics of human genetic diseases. BMC Genomics 2009; 10(Suppl 1):S12; PMID:19594871; http://dx.doi.org/10.1186/1471-2164-10-S1-S12.
105. Oldfield CJ, Xue B, Van YY, Ulrich EL, Markley JL, Dunker AK, et al. Utilization of protein intrinsic disorder knowledge in structural proteomics. Biochim Biophys Acta 2013; 1834:487-98.
106. Tyanova S, Cox J, Olsen J, Mann M, Frishman D. Phosphorylation variation during the cell cycle scales with structural propensities of proteins. PLoS Comput Biol 2013; 9:e1002842; PMID:23326221; http://dx.doi.org/10.1371/journal.pcbi.1002842.
107. Sun X, Rikkerink EH, Jones WT, Uversky VN. Multifarious roles of intrinsic disorder in proteins illustrate its broad impact on plant biology. Plant Cell 2013; 25:38-55; PMID:23362206; http://dx.doi.org/10.1105/tpc.112.106062.
108. Pietrosemoli N, García-Martín JA, Solano R, Pazos F. Genome-wide analysis of protein disorder in Arabidopsis thaliana: implications for plant environmental adaptation. PLoS One 2013; 8:e55524; PMID:23408995; http://dx.doi.org/10.1371/journal.pone.0055524.
109. Wood M, Rae GM, Wu RM, Walton EF, Xue B, Hellens RP, et al. Actinidia DRM1--an intrinsically disordered protein whose mRNA expression is inversely correlated with spring budbreak in kiwifruit. PLoS One 2013; 8:e57354; PMID:23516402; http://dx.doi.org/10.1371/journal.pone.0057354.
110. Costantini S, Sharma A, Raucci R, Costantini M, Autiero I, Colonna G. Genealogy of an ancient protein family: the Sirtuins, a family of disordered members. BMC Evol Biol 2013; 13:60; PMID:23497088; http://dx.doi.org/10.1186/1471-2148-13-60.
111. Coelho Ribeiro MdeL, Espinosa J, Islam S, Martinez O, Thanki JJ, Mazariegos S, et al. Malleable ribonucleoprotein machine: protein intrinsic disorder in the Saccharomyces cerevisiae spliceosome. PeerJ 2013; 1:e2; PMID:23638354; http://dx.doi.org/10.7717/peerj.2.
112. Uversky VN, Oldfield CJ, Dunker AK. Intrinsically disordered proteins in human diseases: introducing the D2 concept. Annu Rev Biophys 2008; 37:215-46; PMID:18573080; http://dx.doi.org/10.1146/annurev.biophys.37.032807.125924.
113. Uversky VN, Oldfield CJ, Midic U, Xie H, Xue B, Vucetic S, et al. Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. BMC Genomics 2009; 10(Suppl 1):S7; PMID:19594884; http://dx.doi.org/10.1186/1471-2164-10-S1-S7.
114. Uversky VN, Roman A, Oldfield CJ, Dunker AK. Protein intrinsic disorder and human papillomaviruses: increased amount of disorder in E6 and E7 oncoproteins from high risk HPVs. J Proteome Res 2006; 5:1829-42; PMID:16889404; http://dx.doi.org/10.1021/pr0602388.
115. Cheng Y, LeGall T, Oldfield CJ, Dunker AK, Uversky VN. Abundance of intrinsic disorder in protein associated with cardiovascular disease. Biochemistry 2006; 45:10448-60; PMID:16939197; http://dx.doi.org/10.1021/bi060981d.
116. Uversky VN. Intrinsic disorder in proteins associated with neurodegenerative diseases. Front Biosci 2009; 14:5188-238; PMID:19482612; http://dx.doi.org/10.2741/3594.
117. Mohan A, Sullivan WJ Jr., Radivojac P, Dunker AK, Uversky VN. Intrinsic disorder in pathogenic and non-pathogenic microbes: discovering and analyzing the unfoldomes of early-branching eukaryotes. Mol Biosyst 2008; 4:328-40; PMID:18354786; http://dx.doi.org/10.1039/b719168e.
118. Xue B, Williams RW, Oldfield CJ, Goh GK, Dunker AK, Uversky VN. Viral disorder or disordered viruses: do viral proteins possess unique features? Protein Pept Lett 2010; 17:932-51; PMID:20450483; http://dx.doi.org/10.2174/092986610791498984.
119. Goh GK, Dunker AK, Uversky VN. Protein intrinsic disorder and influenza virulence: the 1918 H1N1 and H5N1 viruses. Virol J 2009; 6:69; PMID:19493338; http://dx.doi.org/10.1186/1743-422X-6-69.
120. Goh GK, Dunker AK, Uversky VN. A comparative analysis of viral matrix proteins using disorder predictors. Virol J 2008; 5:126; PMID:18947403; http://dx.doi.org/10.1186/1743-422X-5-126.
121. Goh GK, Dunker AK, Uversky VN. Protein intrinsic disorder toolbox for comparative analysis of viral proteins. BMC Genomics 2008; 9(Suppl 2):S4; PMID:18831795; http://dx.doi.org/10.1186/1471-2164-9-S2-S4.
122. Midic U, Oldfield CJ, Dunker AK, Obradovic Z, Uversky VN. Protein disorder in the human diseasome: Unfoldomics of human genetic diseases. BMC Genomics 2009; 10:S12.
123. Vucetic S, Xie H, Iakoucheva LM, Oldfield CJ, Dunker AK, Obradovic Z, et al. Functional anthology of intrinsic disorder. 2. Cellular components, domains, technical terms, developmental processes, and coding sequence diversities correlated with long disordered regions. J Proteome Res 2007; 6:1899-916; PMID:17391015; http://dx.doi.org/10.1021/pr060393m.
124. Xie H, Vucetic S, Iakoucheva LM, Oldfield CJ, Dunker AK, Obradovic Z, et al. Functional anthology of intrinsic disorder. 3. Ligands, post-translational modifications, and diseases associated with intrinsically disordered proteins. J Proteome Res 2007; 6:1917-32; PMID:17391016; http://dx.doi.org/10.1021/pr060394e.

125. Mahmoudabadi G, Rajagopalan K, Getzenberg RH, Hannenhalli S, Rangarajan G, Kulkarni P. Intrinsically disordered proteins and conformational noise: implications in cancer. Cell Cycle 2013; 12:26-31; PMID:23255110; http://dx.doi.org/10.4161/cc.23178.

126. Leach BI, Kuntimaddi A, Schmidt CR, Cierpicki T, Johnson SA, Bushweller JH. Leukemia fusion target AF9 is an intrinsically disordered transcriptional regulator that recruits multiple partners via coupled folding and binding. Structure 2013; 21:176-83; PMID:23260655; http://dx.doi.org/10.1016/j.str.2012.11.011.

127. Wang F, Marshall CB, Ikura M. Transcriptional/epigenetic regulator CBP/p300 in tumorigenesis: structural and functional versatility in target recognition. Cell Mol Life Sci 2013; PMID:23307074; http://dx.doi.org/10.1007/s00018-012-1254-4.

128. Ariesandi W, Chang CF, Chen TE, Chen YR. Temperature-dependent structural changes of Parkinson's alpha-synuclein reveal the role of pre-existing oligomers in alpha-synuclein fibrillization. PLoS One 2013; 8:e53487; PMID:23349712; http://dx.doi.org/10.1371/journal.pone.0053487.

129. Singh PK, Kotia V, Ghosh D, Mohite GM, Kumar A, Maji SK. Curcumin modulates α-synuclein aggregation and toxicity. ACS Chem Neurosci 2013; 4:393-407; PMID:23509976; http://dx.doi.org/10.1021/cn3001203.

130. Maltsev AS, Chen J, Levine RL, Bax A. Site-specific interaction between α-synuclein and membranes probed by NMR-observed methionine oxidation rates. J Am Chem Soc 2013; 135:2943-6; PMID:23398174; http://dx.doi.org/10.1021/ja312415q.

131. Wise-Scira O, Dunn A, Aloglu AK, Sakallioglu IT, Coskuner O. Structures of the E46K mutant-type α-synuclein protein and impact of E46K mutation on the structures of the wild-type α-synuclein protein. ACS Chem Neurosci 2013; 4:498-508; PMID:23374074; http://dx.doi.org/10.1021/cn3002027.

132. Wise-Scira O, Aloglu AK, Dunn A, Sakallioglu IT, Coskuner O. Structures and free energy landscapes of the wild-type and A30P mutant-type α-synuclein proteins with dynamics. ACS Chem Neurosci 2013; 4:486-97; PMID:23374072; http://dx.doi.org/10.1021/cn300198q.

133. Rabe M, Soragni A, Reynolds NP, Verdes D, Liverani E, Riek R, et al. On-surface aggregation of α-synuclein at nanomolar concentrations results in two distinct growth mechanisms. ACS Chem Neurosci 2013; 4:408-17; PMID:23509977; http://dx.doi.org/10.1021/cn3001312.

134. Gurry T, Ullman O, Fisher CK, Perovic I, Pochapsky T, Stultz CM. The dynamic structure of α-synuclein multimers. J Am Chem Soc 2013; 135:3865-72; PMID:23398399; http://dx.doi.org/10.1021/ja310518p.

135. Fantini J, Yahi N. The driving force of alpha-synuclein insertion and amyloid channel formation in the plasma membrane of neural cells: key role of ganglioside- and cholesterol-binding domains. Adv Exp Med Biol 2013; 991:15-26; PMID:23775688; http://dx.doi.org/10.1007/978-94-007-6331-9_2.

136. Schulze-Gahmen U, Upton H, Birnberg A, Bao K, Chou S, Krogan NJ, et al. The AFF4 scaffold binds human P-TEFb adjacent to HIV Tat. Elife 2013; 2:e00327; PMID:23471103; http://dx.doi.org/10.7554/eLife.00327.

137. Dunker AK, Uversky VN. Drugs for 'protein clouds': targeting intrinsically disordered transcription factors. Curr Opin Pharmacol 2010; 10:782-8; PMID:20889377; http://dx.doi.org/10.1016/j.coph.2010.09.005.

138. Rawat A, Kumar D. NMR investigations of structural and dynamics features of natively unstructured drug peptide - salmon calcitonin: implication to rational design of potent sCT analogs. J Pept Sci 2013; 19:33-45; PMID:23208874; http://dx.doi.org/10.1002/psc.2471.