Intrinsically Disordered Proteins

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Digested disorder

Quarterly intrinsic disorder digest (April-May-June, 2013)

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Quarterly intrinsic disorder digest (April–May–June, 2013)

Shelly DeForte,[1,†] Krishna D Reddy,[1,†] and Vladimir N Uversky[1,2,3,*]

[1]Department of Molecular Medicine; Morsani College of Medicine; University of South Florida; Tampa, FL USA; [2]USF Health Byrd Alzheimer's Research Institute; Morsani College of Medicine; University of South Florida; Tampa, FL USA; [3]Institute for Biological Instrumentation; Russian Academy of Sciences; Pushchino, Moscow Region, Russia; [†]These authors contributed equally to this work.

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The current literature on intrinsically disordered proteins is overwhelming. To keep interested readers up to speed with this literature, we continue a "Digested Disorder" project and represent a series of reader's digest type articles objectively representing the research papers and reviews on intrinsically disordered proteins. The only 2 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 issue covers papers published during the period of April, May, and June 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

Intrinsically disordered proteins (IDPs) and proteins with intrinsically disordered protein regions (IDPRs) are found extensively throughout all proteomes, and this typically has a significant bearing on functional and structural considerations.[1-12] Computational and experimental methods for identifying disorder in proteins continue to undergo rapid advancement.[13-15] Also, there is ongoing debate regarding the application of alternate models of protein behavior such as induced fit, conformational selection and fuzzy complexes.[16-18] Despite this activity, the field is still nascent and the implications of intrinsic disorder on function, binding mechanisms, drug-design and disease propensity are often not mentioned or considered in the literature. As an example, performing a search within this quarter on 2 proteins known to be disordered, we find that 8 out of 213 published papers on α-Synuclein and 9 out of 965 papers on amyloid β include the terms "intrinsically disordered protein" or "natively unfolded protein." Here are details and outputs of these searches: ((intrinsically disordered protein) OR (natively unfolded protein)) AND (α-Synuclein) AND ("2013/04/01"[Date - Publication]: 2013/06/30"[Date - Publication]), 8 hits; (α-Synuclein) AND ("2013/04/01"[Date - Publication]: "2013/06/30"[Date - Publication]), 213 hits; ((intrinsically disordered protein) OR (natively unfolded protein)) AND (amyloid β) AND ("2013/04/01"[Date - Publication]: "2013/06/30"[Date - Publication]), 9 hits; (amyloid β) AND ("2013/04/01"[Date - Publication]: "2013/06/30"[Date - Publication]), 965 hits.

Keeping up with the field represents a challenge due to increasing awareness and use of the term and the breadth of coverage across multiple disciplines in the literature. The purpose of this digest is to provide an unbiased and condensed survey of the literature on a quarterly basis. Based on the reported metrics, the first issue of the Disorder Digest[19] gained significant attention of readers (as of November 2013, this article, being published at the end of June 2013, was viewed 435 times), suggesting that the idea of having condensed overview of intrinsic disorder-related literature is welcome.

In the second digest of this series, we cover papers published in April, May and June 2013 using the following search term in PubMed: ((intrinsically disordered protein) OR (natively unfolded protein)) AND ("2013/04/01"[Date - Publication]: "2013/06/30"[Date - Publication]). This search gave 111 hits, and 60 of those papers are reviewed in the digest. Most of the 51 remaining articles were excluded because PubMed searches entries by their Epub date and not print date, and these 2 dates can be wildly different.

As in the previous issue, no special filtering was used except to verify the print date, and exclude those papers not related to the topic. The digest article is structured hierarchically and papers are grouped in several sections: (a) structures of intrinsically disordered proteins (IDPs); (b) functions of IDPs; (c) methods for the IDP analysis; (d) proteomics of IDPs; (e) IDPs and diseases; and (f) 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.

*Correspondence to: Vladimir N Uversky; Email: vuversky@health.usf.edu
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Studies on Structural Properties of IDPs and IDPRs

The study of protein structure becomes significantly more complicated when dealing with IDPs or hybrid proteins containing both structured domains and IDPRs. It is becoming clear that proteins and protein regions cannot be classified as simply disordered or ordered, and instead there is a wide range of structural variability which is often contextual and subject to change.[5,20-23] The dynamic nature of disordered proteins represents a challenge to the experimental paradigm of structural biology because crystal structures may be incomplete or impossible to obtain. However, multiple biophysical methods are available to verify and further classify flexible proteins/regions, including circular dichroism, fluorescence spectroscopy, and NMR. Computational predictions often guide this process.

This multipronged approach was used by Pierce et al.[24] to investigate the yeast scaffold protein Pan1, which was predicted to be mostly disordered by PONDR. Using several experimental techniques, including circular dichroism (CD), tryptophan fluorescence, boiling/pelleting experiments and proteolysis, they confirmed that Pan1 is intrinsically disordered and advocated for its inclusion in DisProt, the database of disordered proteins. The authors verified the formation of Pan1 homodimers and speculated that the disordered regions of Pan1 may impart an advantage, allowing this protein to interact with many different partners.

In some cases, the induced structure of a disordered protein can be captured by crystallography. This snapshot can provide information about the disorder to order transition and molecular recognition. Barandun et al.[25] obtained the crystal structure for the intrinsically disordered prokaryotic ubiquitin-like protein (Pup) bound to its ligase, proteasome accessory factor A (PafA). Pup is a functional analog to ubiquitin that folds into 2 well- resolved helices upon interaction with PafA. They observed that the C-terminus of Pup wraps around PafA, and identified a conserved pocket on PafA where Pup binds. Fluorescence anisotropy measurements showed that binding was low affinity and the thermodynamic driving force for the interaction was provided primarily by one of the helices on Pup.

Conserved regions of disorder may play a role in some protein families and domains.[26] Often times, the domain in question has a highly variable sequence that is consistently disordered. This is true for the C-terminal variable 5 (v5) region of the protein kinase C family, a disordered domain with several functions, including stabilization of the kinase subdomain, participation in auto inhibitory interactions and interfacing with adaptor proteins. Importantly, the high variability of this domain makes it a good isoform specific target. Yang et al.[27] looked at the α-isoform of this domain using CD and NMR. Examination by CD indicated that the v5 domain is mostly disordered with some residual structure, and NMR in the presence of micelles suggested that the v5 region serves as a membrane anchor.

Knight et al.[28] used the intrinsically disordered protein PIR along with a number of differently charged mutants, to investigate the negatively charged ribosomal surface and its influence on protein folding in nascent polypeptides. Positively charged polypeptides tended to be more spatially biased and negatively charged polypeptides exhibited more spatial dynamics. Overall, the ribosome seems to act both as a denaturing agent and a tether, creating an environment with enough flexibility for proper folding, and enough spatial bias to prevent aggregation.

The study of structural evolution in disordered proteins represents a particular challenge. Intrinsically disordered regions tend to evolve and change more quickly than structured regions,[29] but still often preserve their fundamental function. In order to try to untangle the rules of evolution in intrinsically disordered proteins, Ma et al.[30] examined FIgM proteins, a model class of bacterial IDPs, which is functionally conserved but has low sequence similarity. CD revealed that, while all proteins in the family existed in extended conformations, structural specifics varied widely. This indicates that the principles governing the evolution of rapidly changing IDP families are not necessarily bound by structural conservation.

One of the potential functional advantages of IDPs is their increased surface area for interaction. Using atomic force microscopy and NMR analysis, Hashimoto et al.[8] examined the chromatin remodeler FACT from *Drosophila melanogaster*. FACT has an acidic IDPR whose phosphorylation modulates FACT binding to nucleosomes. The authors found that increased phosphorylation does not facilitate binding by increasing structure, but by increasing the number of binding sites and therefore the probability of binding.

Adzhubei et al.[31] wrote a timely review of the polyproline-II (PPII) helix, the only recurring structural class besides α helices and β sheets. The PPII helix is an extended, flexible, left-handed helix without regular hydrogen bonds, which commonly occurs in unfolded proteins and is sometimes mistaken for a random coil. It is particularly prevalent in interaction sites, which often require some innate flexibility. It is currently not considered a standard secondary structure class in the PDB and the authors argue that this hinders recognition and functional understanding of the unique properties of the PPII helix.

McDonald et al.[32] looked at the proline rich (PR) domain of the Sos1 nucleotide exchange factor, which is an important component of signal transduction. This unstructured domain was investigated using a variety of biophysical techniques including tryptophan fluorescence, CD, dynamic light scattering, small angle X-ray scattering, and molecular dynamics. Interestingly, the PR domain adopts a mostly disordered conformation in solution, but a structural transition can be triggered by denaturants, which cause it to form a significant number of PPII helices. This structural tendency was also observed by Elam et al.[33] in the previous quarter and they follow up this quarter with an experimentally determined amino acid propensity scale for the PPII helix using a peptide host-guest system and isothermal titration calorimetry.[34] After this scale was determined, it was used as a predictive tool to check for PPII propensity in representative proteomes. They found that PPII helices could be predicted with reasonable accuracy and PPII propensity was highly correlated with phosphorylation sites and disordered regions.

Analyzing Functions of IDPs and IDPRs

Functions of IDPs and IDPRs are widely varied, as they are influenced by their multiple dynamic conformations. Most known functions of IDPs and IDPRs involve recognition, however many other diverse functions have been identified.

Hub proteins such as p53 are often intrinsically disordered,[35-40] and this ultimately results in several different downstream signaling outputs. Using single-molecule fluorescence resonance energy transfer (smFRET), Ferreon et al.[41] examined the allosteric properties of an intrinsically disordered hub protein, E1A. This study revealed extremely sensitive regulation of E1A interaction with its few interaction partners (CBP/p300, pRb) through either negative or positive cooperativity, which is dependent on E1A interaction site accessibility. Therefore, the specific interaction patterns of E1A dictate subtle differences in molecular conformations of E1A, which ultimately facilitate multiple downstream pathways. This landmark study provides a paradigm of disordered hub protein function through allosteric regulation.

Antibody recognition of an epitope is governed by various factors on both sides of the interaction. An epitope can regulate antigenicity by its structural dynamics, which is facilitated by its disordered nature. Proline isomerization is a known example of this, and Fassolari et al.[42] demonstrated that M1 antibody recognition of E7 oncoprotein is specific to a *cis* isomerization state, which populates only 10% of the species. Proline isomerization occurs at the minute time scale, and thus is the rate-limiting step of antigenicity. This study provides a scenario where subtle structural transitions can dictate rate and specificity of interactions.

It is difficult to experimentally observe dynamic structures in action. Therefore demonstrating functional behavior that is truly independent of structure is often hindered. With the aid of a cleverly designed disulfide bond trap, Housden et al.[43] structurally characterized the interaction between bacteriocin colicin E9 (ColE9) and the *E. coli* trimeric porin OmpF. The intrinsically disordered N-terminal region of ColE9 threads through an OmpF subunit, then threads in the opposite direction of another OmpF subunit. This reveals a relatively fixed epitope that serves to orient TolB to TolA, ultimately triggering colicin entry and subsequent cell death. This 'threading' mechanism provides a justification of how sequence characteristics can directly translate to function independent of structure.

A study of SARS-CoV N protein also uses the disulfide bond trap to great effect. N protein is composed of 3 regions: a N-terminal structural domain (NTD), a flexible linker region (LKR) and a C-terminal structural domain (CTD). The CTD usually exists as a dimer, but can form oligomers at higher concentrations. A study by Chang et al.[44] used a similar disulfide trap combined with NMR spectroscopy to structurally characterize these oligomers, and found that the CTD is the site of oligomerization. Oligomerization is enhanced by phosphorylation of the serine-rich LKR, which lowers the net charge of the protein. This serves as an illustrative example of protein regulation by the IDPR posttranslational modification.

A known function of IDPs is assembly of large complexes. In a review by Malinovska et al.,[45] the authors argue that intrinsic disorder is an essential component of membrane-less intracellular compartments in eukaryotic organisms. IDPs/IDPRs can facilitate large assemblies at the cost of potential aggregation-related phenotypes, and these authors discuss how disordered proteins potentially facilitate cytoplasmic phase separation as well as the critical balance between native states and amyloid states.

The axin scaffolding protein has a long intrinsically disordered region that facilitates phosphorylation of β-catenin by recruiting kinases. A study by Xue et al.[46] used various computational tools to examine the role of intrinsic disorder in this complex, and present a model for function. This model is that of a 'stochastic machine', where random collisions facilitate function as opposed to a structured, compact machine facilitated by coordinated movements. This 'stochastic machine' is dependent on only a few very well-conserved residues.

E3 ubiquitin ligases are in the curious situation of potentially being autoubiquitinated simply by proximity to an E2 ubiquitin-conjugating enzyme, either in *cis* (through its own ligase activity) or in *trans* (another E3 ligase). E3 has multiple mechanisms of avoiding this circumstance, and Fredrickson et al.[47] characterized another avoidance mechanism based on the intrinsic disorder of the N- and C-terminal substrate binding regions of yeast SanI. These regions are noticeably depleted in lysines, and introduction of lysines led to rapid ubiquitin-mediated degradation of E3 both in *cis* and in *trans* independent of substrate binding. Additionally, San1 N- and C-terminal regions recognize exposed hydrophobic residues, and yet are characterized by low overall hydrophobicity. Increasing hydrophobicity of these regions led to *trans* autoubiquitination mechanisms. Taken altogether, this demonstrates how intrinsic disorder has been conserved to prevent autoubiquitination by conservation of sequence and hydrophilicity.

The Bag6-Ubl4A-Trc35 complex interacts with ER membranes tightly to promote ER-associated degradation (ERAD) pathways. However, how this interaction was facilitated was unclear. Xu et al.[48] found that Bag6 has a disordered proline-rich region that homo-oligomerizes, which facilitates interaction of the Bag6 ubiquitin-like (UBL) domain with two ER membrane-associated proteins (gp78 and UbxD8). Formation of these disordered oligomers is essential to downstream ERAD machinery, presumably through facilitating association of gp78 and UbxD8.

Arabidopsis binding protein (BiP) is a chaperone in the ER lumen, and using biochemical and cellular imaging techniques, Srivastava et al.[49] demonstrate that BiP interacts with the sensor/transducer bZIP28. The bZIP28 C-terminal tail mediates this interaction, and this region is predicted to be intrinsically disordered. When the ER is stressed, this interaction is broken up, and bZIP28 localizes to the Golgi, where it proteolyzes and eventually enters the nucleus to constitutively promote transcription of stress response genes. This study provides an example of how a weak interaction facilitated by

disorder can serve as a stress-response switch in plants.

Bacterial pupylation is a functional homolog of eukaryotic ubiquitination. However, the protein modifications are structurally dissimilar. Unlike ubiquitin, prokaryotic ubiquitin-like protein (Pup) is intrinsically disordered in monomeric form and only becomes structured upon binding to its ligase (see a discussion of the crystal structure in the previous section). A review by Striebel et al.[50] encompasses the current knowledge in the field of pupylation, as well as the similarities and differences between eukaryotic and prokaryotic proteasomal degradation mechanisms. The authors argue that some of the unique functions of Pup are likely to be facilitated by its intrinsic disorder.

Intrinsic disorder is commonly found at structured protein tails. The flexible dynamics of these tails facilitate numerous conformations and thus functions. These functions can be classified as both entropic and disorder-to-order transitions, and a review by Uversky[51] provides examples of the many functions of disordered termini. It is argued that the tendency of researchers to simply remove tails for purposes of defining protein structure is flawed. Since these tails often serve essential functions, determining a structure without these tails may exclude essential information.

Membrane proteins often have disordered tails/loops that facilitate protein function. Ihara et al.[52] characterize a fungal TRP channel (TRPGz) that has a cytosolic C-terminal domain (CTD). The CTD was shown to be intrinsically disordered by NMR, and has 2 segments that facilitate function – a region that homo-oligomerizes through formation of a presumed coiled-coil (CC) region, and a region after the CC region that binds phospholipids and regulates channel activation independent of CTD assembly. Interestingly, structural characterization of the presumed CC assembly found a more moderate offset spiral conformation. This assembly is not essential for tetrameric channel formation, but instead is essential to regulation of channel activation by sensing changes in osmolarity and temperature.

In another example of a membrane protein with disorder in the tails that is essential for function, Liu et al.[53] examined selenoprotein S (SelS), an enzyme that has the rare selenocysteine amino acid in its disordered cytoplasmic tail. This residue makes SelS aggregation-prone, which has hindered characterization of enzyme function. The authors made a SelS construct of just the SelS tail (cSelS) and characterized the activity of the wild type and the selenocysteine mutant (U188C). It was found that SelS has thioredoxin-dependent reductase activity as well as low peroxidase activity. Additionally, the selenocysteine of SelS is essential for its reductase activity. This study characterizes yet another member of the class of intrinsically disordered enzymes, a small but growing group of proteins.

An intrinsically disordered linker between the nuclear localization signal (NLS) and the transmembrane region of yeast Src1/Heh1 facilitates nuclear transport. This linker functions by facilitating the interactions between NLS-associated karyopherins and FG-repeat binding sites in the nuclear pore complex. Meinema et al.[54] evaluate the kinetics of karyopherin import as well as efflux of membrane cargos. Based on their data, the authors propose a mechanism where the linker facilitates the interaction, then dodges into the lateral gates to facilitate efflux.

Another example of a disordered region facilitating protein function is mouse synaptic-defective 1A (mSYD1A). Using biochemical techniques and mSYD1A knockout mice, Wentzel et al.[55] determined that mSYD1A is essential to synaptic vesicle docking and transmission. This point of regulation is dictated by a disordered region interacting with multiple binding partners determined using the yeast 2-hybrid technique. Overall, this study provides further evidence that IDPs/IDPRs may serve as regulators of presynaptic assembly and function by dynamically regulating protein-protein interactions and post-translational modifications.

There are also examples of structured features facilitating functions of disordered regions, as disordered protein interactions are often weak. Neuropeptide hormones are often intrinsically disordered, and arise from larger precursors containing a prodomain and an N-terminal signal peptide. Dirndorfer et al.[56] examined deletion and domain-swapped constructs of the synthesized precursors biochemically. The authors demonstrated that the prodomain adopts an α-helical structure, and that formation of this structure promotes import of the hormone region into the ER lumen. The proposed model involved the IDPR being too 'weak' to enter the lumen independently, and the prodomain serving as a helper protein for the hormone to elicit its function.

In a review of alternatively spliced exons, Buljan et al.[57] argues that the phenomenon of alternative splicing of mRNA regions encoding the disordered segments influence protein-protein interaction specificity through various mechanisms. These include alteration of auto-inhibitory kinetics, presence/absence of molecular recognition features, and stability of protein-protein complex formation. This 'rewiring' of interaction networks can lead to profound phenotypic effects on evolution, development, and disease.

Methods for IDP/IDPR Analysis

Computational approaches for the analysis of intrinsic disorder

Research involving IDPs has always been strongly coupled to computational analysis. As these methods mature, so do their predictive and analytical applications. Sequence based predictions of disorder are being used more extensively to classify populations of proteins and new predictive methods are being developed. Molecular dynamics simulations are also being used in increasingly creative ways to extract information about molecular level behavior in IDPs and their partners.

Molecular dynamics simulations

Functions of IDPs do not fit within a static lock and key model, and the mechanistic possibilities are extensive. Molecular dynamics simulations provide a tool with which to computationally examine the dynamics of a protein over time. While computationally intensive and reliant on a series of cumulative approximations that can produce erroneous results, molecular dynamics simulations have nevertheless become indispensable in the study of IDPs and other molecules in motion.

Replica exchange molecular dynamics with solute tempering (REST2) was used by Musiani et al.[58] to study UreG, an intrinsically disordered enzyme from *Helicobacter pylori*. Their model indicated that the catalytic core was somewhat rigid, but the areas involved in protein-protein interaction were largely flexible.

IDPs are generally considered to be able to engage in high specificity interactions, frequently with low affinity. However, it is also possible that flexibility results in many non-specific interactions. Using molecular dynamics simulations, Huang et al.[59] examined the interaction specificity of 35 ordered and 43 disordered protein complexes. Using thermodynamic data combined with molecular dynamics simulations of mutated *vs*. wild type proteins upon interaction with a cognate target, they showed that IDPs are more malleable under perturbation due to mutation, as measured by a smaller free energy change. This reinforces the idea that IDPs may have an advantage in adaptability over more structured proteins, and provides a basis for further studies on specificity.

Several groups explored new ways to quantify binding and residual structure through calculations derived from molecular dynamics simulations. Chu et al.[60] defined the intrinsic energy landscape of 15 homodimers and used this calculation to classify binding-folding dynamics. The intrinsic energy landscape is reflected by the density of states (DOS), a temperature independent distribution description of the microcanonical ensemble, which can be indirectly obtained from molecular dynamics simulations.

The intrinsically disordered peptide, amyloid β (Aβ) features heavily in molecular dynamics studies, due both to its importance, and the abundance of experimental data. Aβ is a small peptide, derived from the amyloid precursor protein (APP), which is best known as a component of amyloid plaques in Alzheimer disease. Chong et al.[61] demonstrated a relationship between conformational entropy and residual structure in the full-length amyloid β peptide. They argued against using a quasi-harmonic approximation of conformational entropy for IDPs, and instead used an energy-based approach which combined molecular dynamics simulations with liquid integral-equation theory.

Fisher et al.[62] described an approach for comparing IDPs with similar sequences and applied this approach to the amyloid β sequences Aβ40 and Aβ42. The ensembles generated from the two similar peptides were compared using a single library of structures and then estimating the weights using Bayesian formalism. The authors found that Aβ42 sampled pre-fibrillar formations roughly an order of magnitude more often than Aβ40.

Xu et al.[63] used replica exchange molecular dynamics as a method to investigate the internal dynamics of zinc-bound amyloid β peptides. Their simulation data showed that zinc-bound Aβ42 was more rigid than Aβ40 at the C terminus, consistent with the NMR results at high and low temperatures.

In order to understand the effects of confinement on IDPs, Rao et al.,[64] performed molecular dynamics simulations on a small central folding portion of amyloid β under 2 conditions, hydrophobic and hydrophilic pores. While turns were enhanced under confinement, hydrogen bonding was not, which indicates a disconnection between structure and hydrogen bonding under confined conditions.

Computational analysis of IDP structures and functions

There are multiple computational tools that can predict IDPRs with reasonably high accuracy, however the potential of these tools to elucidate protein function is not fully realized. Cozzetto et al.[65] explores this in a review on the application of disorder prediction to the study of protein function. Many proteins in UniProt have no experimentally assigned or extrapolated function and the authors suggest that disorder prediction may provide insight into protein function and help fill some of these gaps.

Shirota et al.[66] examined residue pair frequencies in known ordered, soluble proteins vs. known disordered protein sequences. Using co-occurrence scores, they found that hydrophobic and charged residues tended to be disproportionately paired in ordered proteins, while hydrophobic-hydrophobic and charged-charged pairings were suppressed. Disordered residues did not show a suppression of same residue pairings. The authors speculate that certain compositional characteristics are conserved because folding in water requires a balance of hydrophobic and hydrophilic residues

In the category of intrinsic disorder resources, Domenico et al.[67] introduced the MobiDB database (http://mobidb.bio.unipd.it/) which brings together multiple disorder annotation sources into a single place, including disorder predictions, X-ray and NMR data. Currently, the website provides coverage for 4,500,000 sequences, covering all eukaryotic proteomes.

Experimental Approaches for the Analysis of Intrinsic Disorder

NMR

NMR techniques are widely considered to be the gold standard for experimental observation and characterization of disordered proteins. NMR is primarily used to observe the large conformational space that is occupied by a disordered protein. However, this is only one of many potential uses, and other applications of NMR spectroscopy have been developed and theorized. A review by Jensen et al.[68] broadly discusses the use of various NMR spectroscopy techniques in the study of IDPs, as well as the future applications of NMR spectroscopy in observing IDPs/IDPRs.

A review by Kragelj et al.[69] discusses the use of NMR chemical shifts to characterize IDPs in greater depth. Both experimental details and ensemble generating algorithms are introduced. Additionally, this article reviews the various pitfalls associated with only using chemical shift data, especially in observing transient protein interactions.

Traditional multidimensional NMR techniques suffer from limited signal dispersion, which decreases the amount of information regarding the dynamics of disordered regions. Stanek et al.[70] describe a method of utilizing cross-correlated NMR relaxation (CCR) techniques with the crowded ensemble of a NMR spectra of a disordered protein. This method allows for observation of backbone dihedral angles, ultimately allowing for sensitive detection of irregular secondary structural elements in disordered regions.

Understanding the structure and function of a disordered protein through NMR spectroscopy requires optimization and modification of several conditions, both in the polypeptide chain itself (mutations, probes, isotope labeling) and in the environment (solution conditions). Isaksson et al.[71] introduces a pipeline that takes all of these factors in account using a cell-free expression system and novel acquisition and analysis methods. The authors demonstrate a detailed example of this system in practice using B- and T cell receptor domains.

Mass spectrometry

While NMR spectroscopy and X-ray crystallography are the most informative and heavily used methods for understanding protein structure, each has inherent flaws. This is especially evident regarding disordered protein structures, where crystallization requires mostly static structures for diffraction, and NMR is unable to distinguish between different conformational states in an ensemble. Mass spectrometry represents a complementary technique that can be used to generate useful information when examining disordered proteins.

Electrospray ionization - mass spectrometry (ESI-MS) represents a technique that can probe conformational states of a protein, and ESI-MS can be coupled to ion mobility spectrometry (ESI-IMS-MS) to understand additional charge and shape characteristics. A study by Knapman et al.[72] profiles use of this technique in the study of the conformational states of two IDPs: *apo*-cytochrome c, which is a well-understood protein, and *apo*-osteocalcin, which has proven to be a challenge to other structural techniques. Among other observations, ESI-IMS-MS was able to detect a metal ion-induced structural transition of *apo*-osteocalcin to the *holo*-form because of its ability to separate conformational states. Overall, ESI-IMS-MS represents a powerful technique in probing structural transitions of IDPs and IDPRs, not unlike limited proteolysis.[73,74]

Amide hydrogen/deuterium exchange (HDX) is an extremely useful method to measure protein folding/unfolding, hydrogen bonding, change in solvent accessibility upon ligand binding, and epitope mapping. While this can be detected by NMR, mass spectrometry provides a method that does not require isotopic labeling and can be performed on proteins of any size. A review by Balasubramaniam and Komives[75] highlights the use of this technique on detecting IDPs as well as common features of IDPs, such as post-translational modifications, coupled folding and binding, and oligomerization/aggregation.

Crystallization

Crystallization experiments and IDPs represent an inherent conflict, as crystallization requires very stable structures in order for diffraction to occur, and IDPs are very dynamic in nature. However, stabilizing these and other transient structures for the purpose of crystallization is possible, as demonstrated by the crystallization of Pup discussed earlier in this digest. Generally, crystallization of disordered proteins is facilitated by partners called 'crystallization chaperones'. A review by Bukowska et al.[76] summarizes the uses and applications of the most common crystallization chaperones and how they have been used to crystallize otherwise non-crystallizable structures. These approaches generally require other concerted approaches such as protein engineering and additives in order to be successful. Nevertheless, this review addresses an emerging technique of capturing intermediate structures not identifiable by traditional crystallization methods.

Proteomics

Disorder propensity has become an important variable for quantification when examining the overall trends in a group of proteins. This section looks at the large-scale application of disorder analysis to proteomics studies.

Many proteins in *Toxoplasma gondii* are readily acetylated and deacetylated. Xue et al.[77] used various computational tools to examine the structural characteristics of the *T. gondii* acetylome, and found that intrinsic disorder combined with sequence composition facilitates many of these events. While both acetylated and non-acetylated lysines are common in disordered regions, acetylated residues are differentiated by flanking hydrophobic and aromatic residues. This study is yet another example of how the dynamic nature of intrinsic disorder facilitates post-translational events.

Pushker et al.[78] looked at protein disorder trends within and between viral families. They found a wide range of disorder that varied substantially between viral families and within viral families. There was no clear pattern based on the host, nor association between genome size and protein disorder. Within genome families, there was some correlation between genome size and protein disorder, both positive and negative. Ultimately, the amount of disorder seems to depend more heavily on the strategy of the virus than other variables.

Colak et al.[79] looked at 2 different types of disorder in alternatively spliced proteins: flexible disorder, which is conserved in position but not sequence, and constrained disorder, which is conserved both in amino acid sequence and position. They found that alternatively spliced proteins under tissue specific regulation showed the most significant trend toward disorder. Flexible disorder, but not constrained disorder was significantly enriched in tissue-specific alternative exons, while the constitutive exons immediately flanking the tissue specific alternative exons were high in constrained disorder, phosphosites and linear motifs.

As more alternatively spliced proteins are being identified, the distinction between constitutively spliced and alternatively spliced proteins is being questioned. F.C. Chen[80] argues that a number of differentiating factors—including different levels of regulation, distinct biological properties, and enrichment of disordered regions—demonstrate that this distinction is still valid. However, due to the ubiquity of alternative splicing, the line is beginning to blur.

Tekaia et al.[81] analyzed the composition of transcribed megasatellites (large DNA tandem repeats of mostly unknown function) in fungal genomes. They show that about half of the megasatellites are predicted to encode for intrinsically disordered proteins/regions and speculate that these regions might encode for flexible linkers or proteins that participate in multiple interactions.

In order to better understand the evolution of disordered regions, Light et al.[82] explored the relationship between insertions and deletions (indels) in the DNA

of an organism and intrinsic disorder in the encoded protein. Using HMM-HMM pairwise alignments and disorder predictions from Disopred and IUpred, they found that disordered residues are more frequent among indel residues, and are particularly common in long indels which occur more often in the N and C-terminal regions.

Often, intrinsically disordered regions in proteins are involved in protein-protein interactions and molecular recognitions.[14,29,83-94] Many flexible proteins or regions undergo at least partial disorder-to-order transitions upon binding, which is crucial for recognition, regulation, and signaling.[6,21,84,87,94-99] Molecular recognition features (MoRFs) are short IDPRs that undergo a disorder-to-order transition upon interaction with a stabilizing partner. Kotta-Loizou et al.[100] compiled a data set of MoRFs in membrane proteins found in the PDB. They found that MoRFs were mostly located on the cytoplasmic segments, that MoRF containing proteins were implicated in protein binding and cell signaling and that the MoRF binding partners were often putative hubs. Compositionally, they found a statistically significant preference for charged residues and a clear difference in the per-residue surface area as compared with the structured set.

Abrusan et al.[101] surveyed the structural elements of proteins that contain segments derived from transposable elements. They used disorder prediction and in silico structure prediction on a set of DNA transposon proteins and LINE proteins. They found that ORF1 and Gag proteins of LINE and LTR retrotransposons had significantly greater disorder. Their results indicate that transposable elements may incorporate into protein sequences more often than expected, and disorder may play a significant role in a subset of these proteins.

Looking at IDPs/IDPRs in Diseases

Proteins that are fully or partially disordered are implicated in many pathological processes and associated with the pathogenesis of a wide range of human diseases, such as cancer, amyloidoses, various neurodegenerative diseases, cardiovascular disease, etc.[102-116] This is due both to ubiquity of intrinsic disorder in hub and signaling proteins, and also to the tendency of many IDPs/IDPRs toward misfolding and aggregation. In addition, infectious pathogens may utilize protein disorder in host invasion.

IDPs in cancer

The p53 tumor suppressor family is known to function as a hub protein through interactions with various partners. p53 has a conserved and structured DNA-binding domain, which is surrounded by disordered regions. A computational analysis of disorder in the p53 family by Xue et al.[117] reveals that these disordered regions are characterized by high sequence variability. Most notably, the level of intrinsic disorder is positivity correlated with the level of sequence variability, providing further evidence that p53 may be intrinsically disordered in vivo.

IDPs in neurodegenerative diseases

The A53T mutant form of α-synuclein has been identified in some families with the early onset form of Parkinson disease, and is particularly prone to aggregation. In order to investigate the differences between the wild type and mutant form, Coskuner et al.[118] employed molecular dynamics simulations along with thermodynamic calculations. They found a significant alteration in the β-sheet structure compared with the helical structure. They proposed that organic molecules that block the β sheet forming residues may reduce aggregation.

α-Synuclein is generally assumed to exist primarily in monomeric, intrinsically disordered form. However new evidence suggests that it may exist as a stable tetramer under some conditions in the cell. Deleersnijder et al.[119] covered this debate and other issues in a review on the conformational plasticity of α-synuclein. The authors suggested that conformational plasticity may impart a functional advantage to this hub protein, while simultaneously making α-synuclein prone to dysfunction.

One of the pathological hallmarks of Alzheimer disease is aggregation of the intrinsically disordered protein, tau. Larini et al.[120] focused on a small aggregating fragment of tau, spanning residues 273–284. By using ion-mobility mass spectrometry, they were able to obtain a size distribution of early oligomers, while TEM studies provided a time course of aggregation. They then used this information with enhanced sampling molecular dynamics to provide detailed structural information. They were able to show that a point mutation known to increase susceptibility to Alzheimer (ΔK280), shifted the morphology of tau and increased aggregation.

Han et al.[121] published a review dedicated to the myelin-specific proteins, a diverse group of proteins carried in the myelin sheath which interact with lipid bilayers. This group of proteins frequently contains large intrinsically disordered regions that may be implicated in neurological diseases.

Baftizadeh et al.[122] looked in detail at the early stages of fibrillar aggregation in Aβ at residues 35–40 using molecular dynamics. Because the formation of an ordered nucleus is considered a rare event, they opted to use bias-exchange metadynamics, which is beneficial for accelerating rare events. They attempted to investigate the criteria that lead Aβ to a nucleation event. Their results differed from the experimentally observed structure, indicating that the nascent fibril may have a different structure from the extended fibril.

Viral IDPs

NS3 serine protease activity is essential for processing of hepatitis C virus precursor protein. NS3 is intrinsically disordered, and undergoes a disorder-to-order transition upon binding to a structural zinc ion. This binding event is essential for formation of a stable active site and thus protease activity, and this is summarized in a review by Vega et al.[123] This review encompasses various biophysical studies that have led to these findings, as well as development of allosteric modulators of this binding event.

Human α-defensin 5 (HD5) complexes with human adenovirus (hAdV) as part of an immune suppression response; however, this binding site is not well characterized. Flatt et al.[124] used cryoEM as well as molecular dynamics simulations to further elucidate this interaction, and

found that an intrinsically disordered penton base surface loop on the viral capsid stabilizes the complex by interacting with HD5 in multiple conformations. This model is applicable to hAdV that is both sensitive and resistant to defensin activity. Ultimately, this study introduces a model of hAdV sensitivity to human defensins which is regulated by an intrinsically disordered region.

Vaccinia virus, a member of the poxvirus family, requires several components for transcription and replication. One of these units is the H5 protein, and a study by Kay et al.[61] characterize some of its properties. H5 was found to have endoribonucleolytic activity to yield a 3′-OH end, consistent with its role as a transcription terminator. Additionally, H5 has an intrinsically disordered N-terminus which non-specifically binds double-stranded nucleic acids, and can specifically bind partners to the site of DNA replication. The ability of the N-terminus to adopt multiple conformational states is dictated (at least in part) by its dynamic phosphorylation, a common feature of IDPRs.[125-128]

Cotton leaf curl Kokhran virus-Dabawali has a genome which encodes six proteins (V1, V2, C1, C2, C3, C4), and Guha et al.[129] characterized C4, which is assumed to be intrinisically disordered. They confirmed this assumption using prediction analysis, and found that C4 has ATPase and pyrophosphatase activities. ATPase activity was metal-ion dependent, which implies that disorder-to-order transition is required for enzyme activity. This is reminiscient of UreG, an intrinsically disordered enzyme that was briefly discussed earlier in this digest.

Diabetes

Type 2 diabetes is characterized by pancreatic islet amyloid. The major component of this, islet amyloid polypeptide (IAPP), is usually unfolded in monomeric state but can form oligomeric fibrils. These fibrils are a marker rather than a cause of type 2 diabetes, and a review by Cao et al.[130] summarizes what is known about causes of fibrillation and cytotoxic effects. This review discusses the physiological role of IAPP, functional residues, conformations of the monomer as well as the oligomer, and the implications of these on in vivo phenotypes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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