DIGESTED DISORDER

Quarterly intrinsic disorder digest (April–May–June, 2014)

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ABSTRACT

This is the 6th issue of the Digested Disorder series that continues to use only 2 criteria for inclusion of a paper to this digest: The publication date (a paper should be published within the covered time frame) and the topic (a paper should be dedicated to any aspect of protein intrinsic disorder). The current digest issue covers papers published during the second quarter of 2014; i.e., during the period of April, May, and June of 2014. Similar to previous issues, the papers are grouped hierarchically by topics they cover, and for each of the included papers a short description is given on its major findings.

KEYWORDS

database; intrinsically disordered proteins; protein function; structural ensemble; structural proteins

Introduction

This article continues the Disorder Digest series. The goal of this series is to provide an unbiased and condensed survey of the literature on intrinsically disordered proteins on a quarterly basis. As in the previous issues, 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: (1) structures of intrinsically disordered proteins (IDPs); (2) functions of IDPs; (3) methods for the IDP analysis; (4) proteomics of IDPs; (5) IDPs/IDPRs in diseases and as drug targets; and (6) Proteome studies. One should keep in mind that the unambiguous classification of many papers is challenged by the intertwining of the topics they cover. In the 6th digest of this series, we cover papers published in April, May, and June of 2014. We used the following search term in PubMed: (intrinsically OR natively OR naturally OR inherently) AND (disordered OR unfolded OR unstructured OR denatured) AND (protein OR region OR peptide OR domain) AND("2014/04"[PPDAT];"2014/06"[PPDAT]), which returned 79 hits. Fig. 1 represents a word cloud, which shows the most represented words from all the abstracts of papers included in this issue.

Studies on structural properties of IDPs and IDPRs

Structural studies of IDPs are increasingly focusing on the dynamic properties of IDPs. Rather than simply assigning a protein or protein region as ordered or disordered, new methods can illuminate conformational ensembles and binding transitions. Using a combination of NMR and SAXS data, Sterckx et al. were able to obtain a conformational ensemble of the PaaA2 antitoxin from E. coli. They showed that the protein exists in a highly compact conformational ensemble with 2 preformed helices connected by a flexible linker. Linkers are common in multi-domain proteins such as the splicing factor U2AF65. Huang et al. performed an NMR and SAXS study of U2AF65 and demonstrated a large number of electrostatic interdomain interactions facilitated by interdomain linkers. Their results suggest a large conformational ensemble with a wide degree of conformational freedom and a small percentage of preformed RNA-binding domain arrangements. The RelA/SpoT enzyme contains an intrinsically disordered C-terminal regulatory region which sits between 2 domains. Using both CD and bioinformatics methods, Ekal et al. showed that both the length and secondary structure elements are
conserved in Rel proteins, indicating the importance of this region for domain-domain interactions and movement.8

Using several spectroscopic techniques, Perovic et al. investigated the oligomerization of the nacre protein n16.3. Their findings show that n16.3 forms an amorphous protein film, with regions that are intrinsically disordered.9 Pieprzyk et al. demonstrated the homodimerization propensity of the intrinsically disordered N-terminal domain of the Ultraspiracle (Usp) protein from Aedes aegypti. They propose that the dimerization of the N-terminal domain may provide a platform for molecular interactions.10 The Myristoylated Alanine-Rich C Kinase substrate (MARCKS), is an IDP that interacts with actin. This interaction occurs in a conserved and phosphorylated region. Using CD and NMR, Tinoco et al. showed that this intrinsically disordered region gains a slightly ordered structure upon phosphorylation. They speculate that this increased structure might facilitate interaction with the mAb 3C3 antibody.11

Myelin basic protein (MBP) is a well-known IDP, however there is still sparse information on the structural nature of the ensemble in solution. Stadler et al. used SAXS on MBP to provide some insight. Their study shows a high degree of flexibility in the structural ensemble of MBP with a compact core and flexible tails.12 The light chain of microtubule-associated protein 1B (MAP1B) has an intrinsically disordered microtubule binding domain whose mechanism is not well understood. Orban-Nemeth et al. shed some light on this by providing detailed structural information of this intrinsically disordered domain using NMR.13 CD temperature melts were performed by Reichheld et al. to investigate the structure of the cross-linking domains during the assembly of elastin. Solid-state NMR showed a β-strand structure in the coacervate state of elastin, while α-helices were predominant after cross-linking of lysine side chains with genipin.14 Richer et al. investigated the hinge region of type VII collagen (Col7), a protein which plays a critical role in linking different skin layers together. The hinge region is composed of 39 amino acids and interrupts the central collagenous domain. Using CD and NMR spectroscopy, they show that this hinge region is intrinsically disordered. It is not clear whether this region performs an actual hinge function, or provides a flexible interaction site.15

A major task in the study of IDPs is investigating changes upon binding to partner molecules. Saio et al. performed NMR relaxation experiments on 3 trigger factor chaperone molecules in complex with alkaline phosphatase. They show that, while the initial complex was highly dynamic, it became more stable as the
2 proteins became increasingly engaged. They also show that despite this increased stability, multivalent binding maintained an extended and unfolded conformation.\(^\text{16}\) A structural examination by Joseph \textit{et al.} using NMR showed increased stability in the complex formed between LMO4 and DEAF-1, which has a disordered LMO4 binding domain in the absence of LMO4.\(^\text{17}\) Kurzbach \textit{et al.} used a combination of NMR and EPR spectroscopy to examine the binding of osteopontin (OPN) to heparin. They found that the core of OPN lengthens and unfolds upon binding to heparin.\(^\text{18}\) This unfolding-upon-binding model provides an interesting counterpoint to the more common folding-upon-binding transition observed in IDPs. In hopes of elucidating the mechanisms in play during lethal cardiac rhythms, Kopanic \textit{et al.} investigated the structural properties of the C-terminal of connexin45, a component of gap junctions in muscle cells. They found that multiple regulatory partners bound with an intrinsically disordered region in the C-terminal, and propose that this could be a master regulatory domain.\(^\text{19}\) Chandrababu \textit{et al.} used NMR to investigate the folding of the tooth enamel protein amelogenin in the presence of SDS micelles. They confirmed that the complex forms in the N-terminal region and that amelogenin increases in structure upon forming a complex with SDS.\(^\text{20}\)

New studies are bringing us closer to distinguishing between induced fit and conformational selection in studies of IDP binding. Knott \textit{et al.} developed a course-grained simulation model which can capture the binding of IDPs in alternate conformations. They applied this method to the nuclear coactivator binding domain (NCBD) in interaction with both ACTR and IRF-3 to determine the binding mechanism. They found that NCBD binding to either partner occurs via an induced fit mechanism.\(^\text{21}\) Gianni \textit{et al.} describe a kinetic method which distinguishes induced fit from conformational selection and relies on an observation of the rate constant for binding while varying the protein and ligand concentrations in separate experiments.\(^\text{22}\) Using statistical mechanics, calorimetry, and NMR spectroscopy, Krieger \textit{et al.} showed conformational selection in the binding of a fragment of the IDP Gab2 to the growth factor receptor-bound protein 2 (Grb2). A transient polyproline II structure played a critical role in this recognition.\(^\text{23}\) Rogers \textit{et al.} showed that the IDP PUMA, in the BCL-2 family, does not have a similar structure within its unfolded ensemble to the folded structure, therefore showing that it does not use a conformational selection model.\(^\text{24}\) Dogan \textit{et al.} reviewed several recent studies which provided information regarding the kinetic binding mechanisms of IDPs. They found that binding tends to precede folding, however secondary structure elements may pre-form.\(^\text{25}\)

Dry friction arises due to interactions within the protein chain. Echeverria \textit{et al.} used molecular dynamics simulations to explore dry friction in the unfolded state. They found that internal friction arises predominantly from hops between distinct isomeric states in dihedral space.\(^\text{26}\) Wuttke \textit{et al.} and Langridge \textit{et al.} both studied the effects of temperature on IDPs. Wuttke \textit{et al.} used single-molecule Förster resonance energy transfer to examine the chain collapse of 5 different IDPs. They found that all 5 IDPs underwent a chain collapse as the temperature increases, and found that the largest collapse occurred for the most hydrophilic sequences.\(^\text{27}\) Langridge \textit{et al.} looked at the hydrodynamic radius of the IDP p53 with the modulation of temperature. They observed heat induced compaction of p53, in contrast to heat induced unfolding in a model structured protein.\(^\text{28}\) Finally, Hackl used limited proteolysis and mass spectrometry analysis to probe for structured regions in the IDP 4E-BP1,\(^\text{29}\) demonstrating an unusual application of a technique typically used to obtain course grained information about disorder within a protein.

Offenbacher \textit{et al.} demonstrated the substitution of the unnatural amino acid 7-azatryptophan (7AW) into the intrinsically disordered photosystem II (PSII) subunit, and demonstrated that it had no significant impact on oxygen evolution activity.\(^\text{30}\) 7AW has unique, pH-sensitive vibrational frequencies that are sensitive markers of proton transfer. The directed substitution of 7AW into other structural domains may provide a nonperturbative spectroscopic probe.

Soranno \textit{et al.} used single-molecule spectroscopy to examine the behavior of IDPs in crowded environments. They found that IDPs became more compacted with increasing concentration and however the degree of this compaction was dependent on crowder size.\(^\text{31}\)

\textbf{Analyzing functions of IDPs and IDPRs}

Liu and Huang provide a review of the multiple functional advantages provided by intrinsic disorder. These include overcoming steric restrictions in
binding, increasing binding rate, facilitating PTMs, preventing aggregation, and others. In another discussion of the potential advantages of intrinsic disorder, Flock et al. discuss how the high entropy cost of taking on a stable conformation upon binding, can be seen as an advantage instead of a disadvantage. The entropy cost can be modulated through repeating linear motifs, alternative splicing, and post-translational modifications, therefore providing a regulatory advantage. Motlagh et al. discuss allosteric regulation in IDPs, and specifically ensemble based mechanisms of allostery. Alemu et al. investigated the determinants of tissue specific expression variability. Interestingly, they found that IDPs, housekeeping proteins, and highly interacting proteins tended to be less variable, while proteins associated with extra-cellular response and disease tended to be more variable.

IDPRs have been shown to be a key element in many protein-protein interactions. It has been proposed that the intrinsically disordered N-terminal arm of small heat shock proteins (sHSPs) is important to substrate recognition. To investigate this, Patel et al. used replica exchange molecular dynamics simulations along with network analysis and clustering methods and found that there are 2 major conformational forms of the N-terminal arms, and the temperature dependence on the open to closed transition was variable, depending on the protein under examination. Using a variety of biophysical methods, Amartely et al. showed that the central domain of STIL is an IDPR and mediates protein-protein interactions. The centrosomal protein STIL is expressed in proliferating cells and is overexpressed in many types of cancer.

IDPRs can also play central roles in signal transduction networks. Furukawa et al. explore this further by looking at the signaling molecule, stromal interaction molecule 1 (STIM1). STIM1 is interesting because it undergoes an order-to-disorder transition upon oligomerization. They created a mathematical model of the dimerization reaction, and show that Ca^{2+} loss acts as a denaturant, which enables cooperative dimerization. Another protein that is functional while in its disordered state is IκBα. Dembinski et al. investigated mutations that promote disorder-to-order transitions in IκBα and showed that these mutations decreased the efficiency of IκBα in inhibiting NFκB.

Regulation is a key function of IDPRs. Jaramillo-Tatis et al. developed a new SUMO-expression and purification protocol for the preparation of a full length murine SCP1/GIP, and were able to observe the interaction between murine SCP1/GIP and the intrinsically disordered BG21. They show that BG21 has a dual regulatory effect on murine SCP1/GIP that depends on its phosphorylation state.

IDPRs may also be involved in nucleic acid binding or recognition. Using a rational dissection approach, Scognamiglio et al. characterized the protein regions involved in the Nucleophosmin (NPM1, B23)/G-quadruplex complex. In particular, the C-terminal IDPR region of NPM1/B23 contributed significantly to the binding of c-MYC G-quadruplex motif. Tatal et al. discuss what they call Conserved Recognition Elements (CoREs), which are short, structured motifs within long IDPRs that have a unique amino acid composition and tend to bind DNA. Sukackaite et al. investigated the very large IDP Rif1. They showed that conserved region I (CRI) was intrinsically disordered and conserved region II (CRII) was partially folded. Furthermore, they showed that CRII binds cruciform DNA with high selectivity and is therefore a DNA binding domain.

In a point of view article in Epigenetics, Gonzalez-Romero and Ausio discuss the unusual histone H1 variant in Drosophila, called dBigH1. They state that, despite the presence of unusual acidic amino acid patches at the N-terminal end of dBigH1, in contrast to the arginine patches at the N- and C-terminal domains of other histone H1 proteins, the essential requirements of H1 proteins appears to be the presence of a lysine- and alanine-rich intrinsically disordered C-terminal domain.

Several studies were published this quarter regarding the interaction of IDPs at biologic interfaces. Using a variety of modeling techniques, including molecular dynamics simulations, Kurut et al. examined the role of histidine in the charge regulation of unstructured peptides at charged interfaces such as microbial cell membranes. They found that zinc(II)-histidine binding competes with protons and ensures a constant charge distribution over a broad pH interval. Hytönen et al. discuss the role of protein conformation in the regulation of cell-matrix adhesion with a spotlight on the role of IDPRs. They highlight several proteins, such as focal adhesion kinase (FAK) and vinculin, where the IDPRs present in these proteins modulate conformation thereby modulating cell-matrix adhesion. Heparin is a glycosaminoglycan.
component of the extracellular matrix which interacts with many proteins and growth factors at the cell surface and in the extracellular matrix. Peysselon et al. investigated the relationship between the affinity and kinetics of heparin-protein interactions and the amount of intrinsic disorder in heparin binding proteins. They found that lipoproteins and matrisome-associated proteins bound heparin with high affinity, and the association rate was positively correlated with the amount of disorder in heparin binding sites.47

Splice variants are common in stress conditions and intrinsic disorder has been shown to be enriched in alternatively spliced proteins. Rae et al. looked at the splice variants of the stress induced genes AtDRM1 and AtDRM2 and introduced a previously undescribed splice variant of AtDRM1. They demonstrate that all variants are predicted to retain the intrinsically disordered nature of the canonical form.48

Sic1 is an IDP that forms a complex with the Cdc4 subunit of an SCF ubiquitin ligase that. To understand the dependence of the Sic1:Cdc4 interaction on the multisite phosphorylation of Sic1, Liu et al. investigated the conformational properties of the disordered Sic1 N-terminal using single-molecule fluorescence spectroscopy under different salt concentrations. They found that the both the end-to-end distance and hydrodynamic radius decreased monotonically with increasing salt concentration, providing evidence that long-range intrachain electrostatic repulsions are a factor in the conformational landscape of Sic1.49

The small IDP Spd1 is an inhibitor of the large ribonucleotide reductase (RNA) subunit (R1) in yeast. Vejrup-Hansen et al. characterized the homolog Spd2, and showed that it is a CRL4(Cdt2)-controlled IDP that functions with Spd1 in the modulation of RNR architecture.50

Low complexity IDPRs have been shown to exhibit a variety of interesting physical behaviors. Humenik et al. look specifically at the relationship between repeat numbers of low complexity glycine/proline rich regions and the self-assembly rates of spider silk protein. They found that interconnecting at least 2 repeat modules together significantly facilitated the structural transformation from disordered to a β-sheet.51

Kapinos et al. explore the interactions of intrinsically disordered Phe-Gly nucleoporins (FG Nups) with transport receptors (Karyopherins (Kaps)) that participate in nucleocytoplasmic transport. Using surface plasmon resonance, they demonstrate a way to correlate in situ mechanistic, equilibrium and kinetic features of Kaps binding to 4 different FG Nups. They find that a slowly exchanging Kapβ1 phase forms a key constituent with the FG Nups that exists simultaneously in a fast phase, and that this suggests a Kap-centric barrier mechanism.52

Methods for IDP/IDPR analysis

Computational approaches for the analysis of intrinsic disorder

Several new computational tools and methods for the analysis of disordered regions were released this quarter. Xue et al. developed a predictor for short linear motifs (SLiMs) in nuclear hormone receptors called EPSLiM, which integrates multiple methods into a single generalized model.53 A rapid lysine ubiquitination prediction called Rapid UBIquitination (RUBI) was released by Walsh et al.54 In training RUBI, they found that intrinsic disorder was weakly anti-correlated with ubiquitination. The online protein characterization tool cleverSuite was introduced by Klus et al. CleverSuite reports statistics on protein sequences based on their physico-chemical properties. Furthermore, it includes a classifier to allow for the classification of new data sets.55 Dickson and Brooks present a novel strategy to determine structural ensembles for molecular dynamics simulations that uses dynamically defined sampling regions organized in a hierarchical framework.56 Finally, Pryor and Wiener evaluated multiple existing disorder predictors for the detection of intrinsic disorder in membrane proteins.57 They found a clear division between predictors that are effective at evaluating membrane proteins, and predictors that are not, providing useful information for the development of bioinformatics pipelines.

Experimental approaches for the analysis of intrinsic disorder

A special “JMR Perspectives” issue was released by the Journal of Magnetic Resonance this quarter, with several articles focused on the study of IDPs. Konrat offers a general review on the contributions of NMR to the study of IDPs,58 while Novacek et al. provide a review in the on the progress toward optimal-resolution NMR.59 Felli and Pierattelli discuss 13C based methods in the study of IDPs.60 In the Journal of Biomolecular NMR, Pantoja-Uceda and Santoro present
new $^{13}$C-detected experiments that can correlate consecutive $^{13}$C-$^{15}$N groups in IDPs and that also provide recognition of glycine residues, facilitating the assignment process.\textsuperscript{61} Ying et al. discuss the application of band-selective homonuclear (BASH) $^1$H decoupling pulses during the acquisition of the $^1$H free induction decay, and apply this analysis to the IDP $\alpha$-synuclein.\textsuperscript{62} Yuwen and Skrynnikov report a new implementation of the proton-decoupled (PD) CPMG experiment which allows the sampling of $^{15}$N R2 relaxation decay up to ca. 0.5–1s. They discuss the benefits of this implementation on the study of IDPs.\textsuperscript{63} Ball et al. compare 2 different ways of combining NMR spectroscopy with theoretical methods and apply them to determining the structural ensembles for amyloid $\beta$.\textsuperscript{64} Finally, Sara et al. demonstrate the use of novel NMR and EPR techniques to characterize weak protein-protein complexes, such as those seen in IDP interactions.\textsuperscript{65}

Single-molecule force spectroscopy as a method to study IDPs was also covered this quarter. Solanki et al. presented a study using the use of optical tweezers to apply force to single $\alpha$-synuclein molecules to measure their extension, therefore allowing them to characterize the conformational transitions.\textsuperscript{66} Watson and Curtis discuss how an integrated scattering profile can identify differing levels of disorder when using small-angle neutron and X-ray scattering, and apply this method to multiple globular proteins and IDPs.\textsuperscript{67}

**IDPs/IDPRs in diseases and as drug targets**

IDPs are enriched in multiple disease processes and viral proteomes. For example, the human hepatitis C virus proteome is abundant in functionally necessary IDPs and IDPRs, as recently shown by Fan et al. using a variety of bioinformatics techniques.\textsuperscript{68} The paromyxoviral replication machinery is also abundant in IDPs and IDPRs, and the evidence for this, including NMR studies, is reviewed this quarter by Communie et al.\textsuperscript{69}

Neurodegenerative disease-related proteins are also enriched in disorder, and some of the most well studied IDPs are implicated in neurodegeneration. For example, $\alpha$-synuclein is a well-known IDP associated with Parkinson disease, and multiple studies were focused on $\alpha$-synuclein this quarter. Scarlata and Golebiewska provided a review of the evidence linking $\alpha$-synuclein aggregation to oxidation and propose a hypothesis that it is the loss of cellular partners under oxidative conditions that promotes aggregation.\textsuperscript{70} Buell et al. focused on the influence of solution conditions on the nucleation of $\alpha$-synuclein aggregation. They found that secondary nucleation increases dramatically at pH values below 6, suggesting that some intracellular locations, such as endosomes and lysosomes, may facilitate rapid aggregation.\textsuperscript{71} Moriarty et al. looked at $\alpha$-synuclein's participation in copper-mediated pathways of neurodegeneration. They found that N-terminal acetylation abolishes $\text{Cu}^{2+}$ binding, opening new avenues of investigation.\textsuperscript{72} Tavassoly et al. also looked the $\text{Cu}^{2+}$ binding to $\alpha$-synuclein using nanopore analysis with $\alpha$-hemolysin. They found that both the binding of dopamine and $\text{Cu}^{2+}$ to $\alpha$-synuclein caused large conformational changes.\textsuperscript{70}

Tau is another well-known IDP linked to neurodegeneration. Elbaum-Garfinkle et al. used disease-promoting mutants of tau to investigate the link between the microtubule binding of Tau and disease. They found that mutations resulted in an increased affinity for tubulin dimers, but had a negligible effect on binding to stabilized microtubules.\textsuperscript{73}

Huang et al. looked at human proteins with multiple target sites for post translational modifications, which are enriched in the group of IDPs, and found that these proteins were more likely to be involved in disease processes.\textsuperscript{74}

Mozaffari-Jovin examined the protein Prp8k, which has an intrinsically disordered domain. They found that mutations in this IDPR disrupt the regulation of Brr2, which leads to a severe form of retinitis pigmentosa.\textsuperscript{75} Sonnenschein et al. presented a review of several competing views on cancer, including the hypothesis that tumorigenic processes are facilitated by the rewiring of the cell’s protein interaction networks, mediated by IDPs.\textsuperscript{76} Loerch et al. look at the cancer-relevant splicing factor CAPERa and they identify an intrinsically disordered binding partner, called SF3b155. They propose that SF3b155 may offer a platform for coordinated recruitment of U2AF homology motif (UHM) containing splicing factors, such as CAPERa.\textsuperscript{77}

Finally, Pasqualoto et al. propose a molecular modeling approach to the development of a TFPI-like inhibitor called Ambyloamin-X. They find that both the structured and unstructured portions of this protein sustain the inhibitory activity.\textsuperscript{78}
Proteome studies

Mei et al. found that the majority of human autophagy proteins have IDPRs and that many of these IDPRs may be involved in protein-protein interactions. Popelka et al. looked at autophagy proteins in yeast, and conducted several biophysical experiments on the protein autophagy protein Atg3, and showed that it contains multiple IDPRs. From this point, they expanded their search using disorder prediction to all Atg proteins in yeast and found that about half of them contained significant disorder.

Peng et al. provided an analysis of 3411 ribosomal proteins and found that they were enriched in intrinsically disordered which had distinct characteristics in terms of evolutionary conservation and involvement in protein-protein interactions. They conclude that intrinsic disorder is not just present, but also necessary to the proper functioning of the ribosome.

Mitic and Pavlovic et al. published a 2 part study looking at the incidence of disorder in T-cell epitopes. They found that epitopes presented by human leukocyte antigens class-I or class-II were predicted to be 2.5 times higher in ordered than disordered regions. They also found that high affinity binding epitopes were more hydrophobic than low affinity binding epitopes.

Aiken et al. performed a genome-wide analysis of the C-terminal tail regions (CTTs) of microtubules (MTs) in yeast. They used a systematic collection of mutants in budding yeast and found that CTTs are not essential, but the loss of either α or β CTTs sensitizes cells to MTT destabilizing drugs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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