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Digested disorder

Quarterly intrinsic disorder digest (July–August–September, 2013)

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The current literature on intrinsically disordered proteins grows fast. To keep interested readers up to speed with this literature, we continue a "Digested Disorder" project and represent a new issue of reader's digest of 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 third quarter of 2013; i.e., during the period of June, July, and September of 2013. Similar to previous issues, 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

This article continues Disorder Digest series started last year.[1,2] 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 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.

In the third digest of this series, we cover papers published in July, August and September of 2013. Recognizing that in the related papers intrinsically disordered proteins (IDPs) and IDP regions (IDPRs) may be referred to a number of different ways, we extended our search criteria this quarter. We also changed our date filtering to only include print dates, with the assumption that in most cases, e-publications will be followed by print publications. 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 ("2013/07"[PPDAT]: "2013/09"[PPDAT]), which returned 89 hits. We find that "intrinsically disordered protein" has become the most common way to refer these proteins, as demonstrated by the fact that the search: (intrinsically disordered protein) AND ("2013/07"[PPDAT]: "2013/09"[PPDAT]) returns 68 hits, only 21 fewer than our complete search which has a full 64 different possibilities, however there are still a number of papers which use different terminology, and we have endeavored to be as complete as possible. After filtering our 89 hits to exclude papers that are not relevant to the topic, we have covered 79 articles here.

Phosphorylation of intrinsically disordered regions features heavily in this issue, and evidence is accumulating that the phosphorylated IDPRs are often critical for regulation with long-range structural and functional consequences. Papers on IDPs in hepatitis C, hepatitis B, HPV, HIV, and polio came out this quarter and the α-synuclein and dopamine interaction is explored in several papers. The intrinsically disordered regions of the cytoskeletal FtsZ have received some attention from multiple groups this quarter. The IDP chaperonin GroEL and its various substrates were discussed in several papers. Two groups established that IDPRs become more compact in acidic conditions, and this is likely to lead to increased understanding of the mysterious structural properties of these proteins in the future. Molecular dynamics (MD) simulations is a technical focus of the computations side of this issue. As IDPRs are highly dynamic and occupy various conformational substates, MD simulations represent a computational means to understand the conformations of an IDPR over time. Additionally, several groups combined principles of MD simulations with various experimental approaches in order to make more definitive conclusions regarding IDPR dynamics.

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Structural Analysis of IDPs and IDPRs

In the 20th anniversary of the journal *Structure*, Forman-Kay and Mittag[3] provide a comprehensive history of the field of intrinsically disordered proteins. They describe the inherent properties of an IDPR based on 5 categories: sequence, forces, structure, function, and evolution. Overall, the authors review how these properties of IDPRs are shifting the protein structure-function paradigm, and how this paradigm shift is changing our understanding of the complex regulation of the biological systems at the molecular level.

Observing the interactions of IDPRs with membrane interfaces remains a challenge. A study by Lopez-Montoro et al.[4] introduced a method of doing this using *E. coli* ZipA, a protein essential for cell division. ZipA has a transmembrane α-helical N-terminus, a globular C-terminus, and a flexible linker region. As a model, the authors used Langmuir monolayers with an air/water interface, which is believed to reliably mimic the intracellular membrane environment. At low grafting densities (i.e., less crowded membrane environment), the linker regions adopted a random-coil conformation, and at higher grafting densities, the conformation changed to a more rigid brush-like state. This study provides evidence of a model where ZipA explores a large portion of conformational space to seek out its partner, FtsZ.

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel are the basis of cystic fibrosis. In comparison with other chloride channels CFTR possesses a unique R-region, which is an IDPR that is dynamically phosphorylated at ten sites by multiple kinases. Bozoky et al.[5] reviewed the various structural changes induced by phosphorylation, and analyzed the effects of these phosphorylation events on inter- and intra-molecular interactions. Overall, the authors summarized the evidence supporting a role of the R-region functioning as a hub protein, interactability of which is changing based on the phosphorylation status.

Osteopontin (OPN) is an IDP that is involved in cancer metastasis. Kurzbach et al.[6] examined OPN using a combination of both NMR and electron paramagnetic resonance (EPR), and found that this protein does not exist solely as a wholly extended IDP, but rather samples a number of conformational states, including cooperatively folded structures unfolding of which is characterized by sigmoidal profiles. This study adds to the literature regarding a heterogeneous spectrum of order *vs*. disorder, and demonstrates that this spectrum exists even in solely unbound states.

E. coli GroES is a heptameric co-chaperonin that complexes with GroEL, and performs much of the folding functions in the cell. Chandak et al.[7] used 2D NMR combined with DMSO-quenched exchange methods to examine the hydrogen/deuterium-exchange kinetics of the heptameric GroES. The authors found that while GroES has globular regions, significant portions of this protein were disordered. The H/D-exchange behavior was not consistent with simple 2- or 3-state unfolding, therefore, the authors proposed a model where the heptamer dissociates into smaller oligomeric intermediates which were not proposed in previous models.

Hendra virus (HeV) nucleoprotein (N) has an intrinsically disordered C-terminus (N_{TAIL}) that interacts with polymerase co-factor phosphoprotein (P) C-terminal X domain (XD) to recruit viral polymerase. A study by Communie et al.[8] used a combination of NMR spectroscopy, X-ray crystallography, and electron microscopy to characterize this interaction, and found that N_{TAIL} undergoes a disorder-to-order transition upon binding to XD. Additionally, this interaction was found not to rearrange the nucleocapsid to increase accessibility of the viral genome to polymerase, indicating that there may be some other factor at play.

At least 75% of intrinsically disordered proteins are polyampholytes, including both positive and negative charges. Using atomistic simulations, Das and Pappu[9] demonstrated that net charge per residue (NCPR) is not an ideal parameter for describing sequence-ensemble states of IDPs. Instead, the authors proposed that sequence-ensemble relationships can be described using additional parameters such as fraction of charged residues (FCR)—also known as total charge—and κ, which is based on linear sequence distributions of differently charged residues. This study provides an alternative basis of sequence-ensemble relationships of IDPs and may change the paradigm of understanding IDP structure and function.

PFMG1 is a Japanese pearl oyster gene responsible for creating pearls in response to an external threat. Perovic et al.[10] determined that recombinant PFMG1 is an intrinsically disordered protein that has a region with homology to an EF-hand Ca^{2+} binding domain. Using various biophysical techniques such as circular dichroism and atomic force microscopy, the authors found that PFMG1 forms amorphous, variably sized oligomers and films, and oligomerization is enhanced by Ca^{2+}.

The function of apolipoprotein (apo) A-1, the main protein component of high-density lipoprotein (HDL), is greatly aided by its flexibility. Phillips[11] reviewed apoA-1 structure and how it aids in interactions with water, lipids, and proteins. The N-terminal two-thirds of apoA-1 are characterized by amphipathic α-helices that are able to transition from order to disorder rapidly, and the C-terminus is intrinsically disordered but gains α-helical structure upon binding to lipid. Overall, the dynamic nature of apoA-1 contributes to the heterogeneous population of HDL species, and Phillips argued that understanding the dynamics of apoA-1 could lead to targeted therapeutics regarding HDL functionality.

De novo protein synthesis, especially regarding IDPs, is an extremely new field. In a study by Rydberg et al.,[12] polypeptides were designed where different sequences that were intrinsically disordered were able to form helix-loop-helix heterodimers upon binding. The authors introduced a catalytic site in one of the subunits that was inactive in monomeric state, but became catalytically active upon folding. Finally, the authors described the forces that are responsible for binding, recognition, and discrimination. This study represents a test of modern knowledge of de novo protein synthesis regarding IDP coupling, and provides a basis for larger and more complex proteins to be designed.

A major problem with using steroid hormones as therapeutics is the inability to control off-target effects. Multiple areas of research implicate that the intrinsically disordered N-terminus of AF1 is an essential modulator of steroid receptor activity, and a review by Simons Jr. and Kumar[13] briefly summarized these studies. Additionally, the authors argued that understanding disorder can be used to create more targeted therapeutic approaches.

While many IDPs undergo a disorder-to-order transition upon binding, some IDP preserve significant amount of disorder even in the bound state, illustrating an interesting phenomenon, often called "fuzziness". Song et al.[14] examined the fuzziness of the transactivation domain of Ewing's Sarcoma oncoprotein family (EAD) using a combination of molecular simulations and transactivation assays. The authors found that fuzzy interactions between EAD and a generic globular target is a result of polycation-π contacts between EAD and basic residues on the target. This mechanism, combined with the advantage of EAD conformational entropy, further supports the concept of fuzziness and demonstrates a structure-independent mode of IDP interaction.

Macromolecular crowding by neutral polymers attempts to model cellular environment in order to help better understanding of how molecules act as a result of their surroundings. Sotomayor-Perez et al.[15] used this principle to analyze the activity of RC_L, a calcium-binding protein, in the presence of a crowded environment. In previous studies, RC_L is shown to be intrinsically disordered in its native state, and gains a compact, folded structure in its holo-form. The crowded environment did not significantly change these states; rather, the crowding agent Ficoll70 stabilized these states. Additionally, the crowded environment increased the affinity of RC_L for calcium. Overall, the authors argued that the crowded environment in bacteria favors a disordered state of some IDPs that facilitates their secretion to the extracellular environment, where they can undergo calcium-induced structural changes.

p21-activated kinases (PAKs) are separated into 2 groups, type I (PAK1, PAK2, and PAK3) and type II (PAK4, PAK5, and PAK6), based on their domain architecture and regulation. PAK4, the most studied member of the group II PAKs, has an autoinhibition mechanism triggered by an intrinsically disordered N-terminal region. Using various biophysical and structural techniques, Wang et al.[16] found that PAK4 autoinhibition is completely different from the autoinhibition of group I PAKs by means of a disorder-to-order transition in the N-terminal region. Overall, this study highlights that targeting of group II PAKs requires a totally different methodology than group I PAKs.

The light-sensitive activity of rhodopsin, a G protein coupled receptor (GPCR), depends on the activation transducin (Gt), a heterotrimeric G protein. Using a combination of molecular dynamics and Fourier transform infrared (FTIR) spectroscopy, Elgeti et al.[17] attempted to further understand rhodopsin/Gt dynamics. The authors found that a cytoplasmic loop (CL3) between transmembrane regions 5 and 6 is intrinsically disordered, though it is found to be helical in bound crystal structures of rhodopsin. The C-terminus of the α-subunit of Gt (GαCT) interacts with CL3, while the C-terminus of Gγ (GγCT) does not. Since both subunits interact with rhodopsin, the authors proposed a sequential model of GPCR/G protein interaction, where γ subunit interacts first and then the α-subunit interacts more specifically, due to the interaction with CL3.

The meta-structure approach is a recently developed conceptual framework where individual residues are evaluated based on compactness and secondary structure, and this is represented as an intricate network of interacting residues. A study by Geist et al.[18] used this framework to understand structural changes in IDPs under acidic conditions, as IDPs are often observed to gain compactness upon lowering in pH. The authors found that using only primary sequence information, the meta-structure approach predicted IDP compactness upon lowering of pH, and they confirmed their findings using NMR to analyze the structural changes of BASP1 and Tcf4. Overall, this study provides a basis for sequence-based prediction of structural changes based on pH.

The interaction between globular KIX (a CREB domain) and the transactivation domain of c-Myb, which is an IDP, is initiated by a disorder-to-order transition. A study by Giri et al.[19] characterized the transition state of this interaction using Φ-value analysis, which involves designing several site-directed mutants. The authors found that the transition state is cooperative and resembles the native state. Additionally, the binding rate constants did not correlate with c-Myb stability, and the authors suggest that the dynamic nature of disorder, by itself, does not increase the rate of interaction.

Notaden bennetti frogs and *Euperipatoides* sp. velvet worms generate extremely similar glues in terms of phenotype. Graham et al.[20] used various biochemical techniques to analyze the 2 major glue proteins, and concluded that these proteins, although different in composition, are likely to be intrinsically disordered. The data hints that these 2 different species glue generation may occur through similar mechanisms, and that this suggests convergent evolution.

IDPRs are known to be more accessible to post-translational modifications such as phosphorylation than ordered regions.[21-23] A study by Grosely et al.[24] examined phosphorylation in the disordered C-terminus of connexin 43, which regulates intercellular communication via gap junctions. Using CD and NMR in combination with Asp-substituted phospho-mimetics, the authors found that phosphorylation affects helical propensity proximal and distal to the site of modifications, due to changes in backbone flexibility and conformational preference, as opposed to stabilization of a helical region.

The C-terminus of the WASp-interacting protein (WIP) is responsible for interaction with WASp, which is critical for its activation and degradation, among other functions. A study by Haba et al.[25] utilized NMR to characterize the WIP C-terminus as IDPR, with short regions of increased helix propensity. Overall, this study is the first biophysical study of WIP C-terminus in an unbound state, and has implications for multiple functions of this interesting and important protein.

PreCol-NG is a collagenous protein of mussel bypass thread. Using circular dichroism and fluorescence spectroscopy, Heim et al.[26] characterized the C-terminal flank region of preCol-NG,

and found that it was intrinsically disordered, even at increased temperature. However, in the presence of small unilamellar vesicles (SUVs), preCol-NG underwent a transition to a β-sheet structure, and the authors suggest that this flank region is essential to byssal protein assembly of mussels.

Functional Analysis of IDPs and IDPRs

It is commonly thought that disordered regions are well suited to high-specificity/low affinity interactions,[27,28] however, the specific mechanism has been difficult to characterize experimentally. Using quantitative anisotropy titrations, Li and Lucius[29] looked at the ATP dependent prokaryotic chaperone, ClpA, which is known to bind both a variety of specific tags as well as general regions with little or no structure. The authors analyzed the interaction of ClpA with the 11-amino acid SsrA tag alone, longer disordered region containing no specific tag sequence alone and with the disordered region containing SsrA tag. This analysis revealed that ClpA possesses superior binding affinity to a long disordered substrate containing SsrA tag compared with SsrA alone, or the unstructured region alone. They hypothesize that the additional non-specific contacts increased affinity.

Mao et al.[30] looked at the regulation of Atg29, a component of the Atg17-Atg31-Atg29 complex which is involved in autophagy initiation. They identified intrinsically disordered regions in Atg29 through observation of missing electron density, sensitivity of the C-terminus to proteolytic cleavage and computational disorder prediction with IUPred. This region is regulated by phosphorylation, upon which, Atg29 undergoes a significant conformational change which plays a key role in its regulation.

The NMDA sensitive glutamate receptor is inhibited by zinc binding in the extracellular N-terminal region, which is regulated by phosphorylation of disordered residues in the C-terminal intracellular region. The exact mechanism of this is unknown; however Choi et al.[31] found that mutating the proline residues in the intrinsically disordered region cancelled the regulatory effects. Single molecule fluorescence and ensemble biophysical methods showed that proline mutation did not eliminate the intrinsic disorder, but changed the conformational dynamics. This indicates that the phosphorylated IDPR is important in allosteric regulation and that the necessary conformational dynamics are finely tuned, and not simply random.

In order to support the hypothesis that disordered linkers may play an allosteric role in conformational switching, Akimoto et al.[32] looked at a disordered linker region in the regulatory subunit of protein kinase A. They observed that this linker region assisted cAMP in disrupting the kinase subunit A and subunit C interface, and speculated that disrupting large protein-protein interfaces by assisting small molecules may be a general function of disordered linkers.

Two papers came out this quarter exploring the function of the intrinsically disordered region of FtsZ, a cytoskeletal protein required for bacterial cell division. The intrinsically disordered linker in the C-terminal region of FtsZ has been a largely unexplored region due to its absence in crystal structures and lack of sequence conservation. Gardner et al.[33] verified the disordered status of the region with CD and trypsin proteolysis, and also showed that the protein performed normally under a variety of sequence changes, as long as the region remained disordered and intact. Buske and Levin[34] also found that the disordered linker was critical for FtsZ assembly and confirmed that the linker was robust under sequence changes, but disorder was required. They hypothesize that the disordered linker allows FtsZ to associate with the membrane while simultaneously interacting with itself and modulatory proteins.

Using a systems wide computational analysis, Ng et al.[35] looked for defining features in proteins which are more efficiently ubiquitylated after heat shock. They found that IDPs were disproportionately ubiquitylated and proposed that IDPRs are more easily recognized in protein quality control pathways.

Employing a 20S proteasomal degradation assay, as well as disorder prediction, Adamovich et al.[36] determined that the transcription coactivator PGC-1α is intrinsically disordered, and is protected from degradation through binding with NQO1, a protein which also protects a number of other partially or fully disordered proteins from degradation by 20S.

Nunomura et al.[37] examined the disordered coiled structure in the GHP domain of protein 4.1G, a membrane skeletal protein. Upon binding to Ca^{2+}-saturated calmodulin, the disordered domain becomes compact and stable resulting in a dramatic conformational change. This in turn causes steric hindrance of 4.1G FERM domain and inhibits a number of interactions with membrane and its membrane associated binding partners.

The nucleolar scaffold protein WDR46 has intrinsically disordered N- and C-terminal regions. Hirai et al.[38] found that these disordered N- and C-terminal regions are primarily responsible for nucleolar localization and serve as binding sites for a number of nucleolar proteins. The authors propose that interactions via disordered regions (as opposed to sequence dependent transport) may be one of the significant factors in nucleolar localization.

Ameloblastin (AMBN) is an intrinsically disordered matrix protein involved in forming tooth enamel. Walt et al.[39] used a variety of experimental techniques including chromatography, analytical ultracentrifugation, transmission electron microscopy, and atomic force microscopy to demonstrate the self-assembly of AMBN into ribbon like structures. Deletion experiments along with NMR showed 2 key structured regions in the N-terminal segment which are necessary for self-assembly.

As another example of a functional intrinsically disordered domain, Valsecci et al.[40] examined the plant specific TCP8 transcription factor in *Arabidopsis thaliana*. The authors determined that this transcription factor has 3 IDPRs and conformed phosphorylation of Ser in these regions by mass spectrometry. Yeast 2-hybrid assays reveal that the C-terminal IDPR corresponds to a transactivation domain, and removal of this C-terminal domain caused a failure of TCP8 to oligomerize. Therefore, it appears that this disordered domain has several key functions.

Cumberworth et al.[41] reviewed promiscuity in IDPs with a focus on interaction networks. They discussed the formation of dynamic macromolecular structures through interfacing with key regions in disordered proteins, namely MoRFs (molecular recognition features), SLiMs (small linear motifs) and LCRs (low complexity regions). Also discussed is the importance of IDPRs in signaling through regulatory switches and recognition of IDPRs in protein quality control.

Prothymosin α (PTMA) is a conserved, highly acidic IDP expressed in male gonads, which adopts a random coil conformation.[42,43] Using a confocal approach, Ferrara et al.[44] verified expression of this protein in mammalian spermatozoa. The authors suggest that the unique composition of this protein may enable viability in a number of different cellular environments and the ability to engage in a number of different functional behaviors.

Frye et al.[45] used electron microscopy to study the structure of early mitotic inhibitor 1 (EM1), a protein whose inhibitory behavior is necessary for coordination of DNA synthesis and mitosis. The authors found that the intrinsically disordered D-box, linker and tail elements play a significant regulatory role by binding multiple sites and shutting down various functions of the anaphase-promoting complex/cyclosome.

The glycosylase NEIL1 contains an intrinsically disordered C-terminal domain, not conserved in its prokaryotic counterpart. Hedge et al.[46] explored whether this region was necessary for proper function and found that while it was dispensable in vitro, catalytic activity was halted in vivo in the deletion mutant. By examining the fluorescence spectra of the wild type and deletion mutants, and observing the C-terminal domain through small angle X-ray scattering, they deduced that this disordered C-terminal domain stabilizes the rest of the protein through electrostatic interactions, while remaining flexible, and may provide an important interaction interface.

Khan et al.[47] examined the functional necessity of the N-terminal disordered domain of Hop1, a component of the synaptonemal complex in *Saccharomyces cerevisiae*. Using a number of experimental techniques, including CD, fluorescence, dynamic light scattering and atomic force microscopy, the authors determined that this disordered domain was required for spore formation and meiosis, but was not required for DNA binding.

Methods of IDP/IDPR Analysis

Molecular dynamics simulations

Molecular dynamics (MD) simulations provide a means to understand the dynamics of IDP/IDPR conformational ensembles. However, a challenge has been related to understanding of a set of time-scale parameters to properly represent the equilibrium of an IDP as well as unfolded ensembles of natively ordered proteins. A study by Das et al.[9] described a method of generating diverse conformational ensembles, using a number of physicochemical properties. The authors applied their approach to the folding kinetics of 15 proteins, and found that their approach closely matches experimental findings. This approach of generating unfolded ensembles has implications for understanding protein folding, misfolding, and non-folding.

Amyloid β (Aβ) is an IDPR that forms oligomers implicated in Alzheimer disease. Structural studies of monomeric Aβ are difficult due to its flexibility. To overcome this, Rosenman et al.[48] used a combined MD/NMR approach to study the oligomerization/aggregation kinetics of 3 species of Aβ. The authors demonstrated that distinct regions within the peptide sample certain conformations using MD simulations, and confirmed their data with NMR studies. Monomeric Aβ is the building block for more pathogenic oligomeric species and this detailed structural study provides the basis for more targeted therapeutic approaches.

While 2D NMR is a valuable tool in understanding ligand binding to IDPs/IDPRs, quantitatively evaluating the contributions of ligand binding to chemical shifts is complicated. Dibenedetto et al.[49] described a tool to facilitate understanding in this field based on MD trajectories, and then applied this method to the α-synuclein-dopamine interaction. Using this tool, the authors were able to determine preferential binding residues. Overall, the described tool represents a useful method for understanding 2D NMR chemical shifts for protein-ligand interactions.

Secondary structure content can be monitored using the amide-I band in FTIR spectroscopy. These data are often paired with MD simulations to provide the greatest possible understanding of secondary structure. However, IDPs cannot be reliably analyzed with this approach. A study by Sethi et al.[50] developed a Bayesian framework to infer properties of IDP ensembles using MD simulations and FTIR spectra. This framework was rigorously validated, and the thermodynamic weights were reproducible and comparable to measured chemical shifts.

Computational analysis of IDP structures and functions

IDPRs tend to be more exposed and are therefore a common site for protein phosphorylation.[21,51] However, it is becoming clear that disordered regions play an important role in regulation by phosphorylation for a myriad of reasons that extend beyond their accessibility. Nishi et al.[52] explored the intersection between protein phosphorylation, intrinsic disorder, and protein binding using bioinformatics analysis on a large set of protein complexes. The authors found that about one quarter of disordered interface sites are phosphorylated, and through the examination of specific cases, suggested that phosphorylation may disrupt functional disorder-to-order transitions.

In a study by Lyle et al.,[53] a parameter Φ is introduced to analyze MD simulations of IDPs. This parameter Φ represents the degree of conformational heterogeneity of either the same polypeptide at different conditions, or different polypeptides in the same conditions. The authors described the development, validation, and uses of parameter Φ in understanding disorder-to-order transitions as well as de novo polypeptide design.

IDPs are known to play roles in most, if not all, cellular pathways. A study by Peng et al.[54] used multiple disorder predictors and other computational tools to understand the prevalence of intrinsic disorder in programmed cell death pathways (PCD), a

cellular suicide mechanism which serves to maintain cell quantity for various reasons. As expected, the authors found that intrinsic disorder is widespread throughout the PCD interactome, and show the various PCD processes that are impacted by intrinsic disorder.

The CFTR R domain was previously addressed in this digest in terms of phosphorylation (in Structural Properties of IDPs section). A study by Sebastian et al.[55] used various genomic analyses to understand both the origin and evolution of this domain. The authors found that the R domain originated ~550–650 million years ago at the base of the Gnathostome lineage, and began as a non-coding intron before becoming a coding exon. From there, the R-domain demonstrated evolutionary changes demonstrating conservation of function. This study represents an evolutionary analysis of an IDP, and shows a method regarding how an IDP originated and evolved over time.

The coupled folding and binding phenomenon of IDPRs is slowly beginning to be understood in terms of physicochemical properties. A study by Wong et al.[56] used various computational tools to determine the importance of polar residues, and found that polar interactions are uniquely important in coupled folding and binding. As a characteristic of coupled folding and binding is the high interaction specificity, the authors propose that polar interactions are a potential reason for this.

Seeger and Rice[57] used various computational tools to predict intrinsic disorder in the kinesin superfamily, which is important for intracellular trafficking. The authors found that disordered regions lie outside of the motor domains and are generally tails. Additionally, the disordered regions are post-translationally modified and/or responsible for binding cargo, suggesting that disorder dictates functional specificity between kinesins.

Experimental Approaches in IDP/IDPR Analysis

NMR

NMR remains the best way to experimentally observe and characterize the structural dynamics of IDPs and IDPRs. The most commonly used isotope is ^{13}C because ^{13}C chemical shifts have much larger dispersion than ^{1}H chemical shifts. Pantoja-Uceda and Santora released two papers this quarter that introduce new methods for ^{13}C-detected NMR that have particular applicability to intrinsically disordered proteins and regions. In a paper published in the Journal of Magnetic Resonance, these authors introduced a collection of pulse sequences to classify CACO and CON spectra by amino acid residue type.[58] In the Journal of Biomolecular NMR, Pantoja-Uceda and Santoro proposed a pair of 3D correlation experiments that use the 3D hNcocaNCO and hnCOcaNCO spectra in combination with the 2D CON spectrum and demonstrate this method on Nupr1, a 93 residue IDP.[59]

With a focus on obtaining atomic resolution characterization of large IDPs, Novácek et al.[60] demonstrated a new ^{13}C-NMR protocol and applied this method to microtubule associated protein 2c. The authors were able to determine the complete backbone, and 98.2% of side chain chemical shift assignments.

Leftin et al.[61] used 2-dimensional ^{13}C separated local-field NMR to look at the membrane interactions of the intrinsically disordered protein α-synuclein. Their observations indicated that α-synuclein induces a thinning of the lipid bilayer and increase in the phospholipid cross sectional area. This annealing process may alter the regulation of synaptic membrane fusion and be implicated in the pathology of Parkinson disease.

Roche et al.[62] explored the effect of hydrostatic pressure on the NMR spectrum of disordered proteins by using high pressure NMR on α-synuclein. The observations of these authors suggest that pressure dependence of the NMR spectrum in disordered proteins is modulated by long range interactions, in contrast to folded proteins, however the nature of these interactions requires further investigation.

To illuminate the properties of disordered substrates upon binding to the chaperonin GroEL, Nishida et al.[63] used stable isotope-assisted nuclear magnetic resonance (NMR) spectroscopy to observe both α-synuclein and chemically denatured bovine rhodanese in complex with GroEL. The obtained results indicate that both GroEL substrates remained highly mobile in complex.

Libich et al.[64] introduced a novel approach for probing the NMR invisible state of a dynamic complex using four complementary NMR experiments: lifetime line-broadening, dark-state exchange saturation transfer, relaxation dispersion, and small exchange-induced chemical shift. They employed this method on the GroEL/amyloid-β complex and were able to verify 2 primary sites of interaction that coincide with the predicted consensus sequences. Their results indicate that amyloid-β remains disordered upon binding to GroEL, and they suggest that this provides an advantage for GroEL function.

Amata et al.[65] demonstrated the usefulness of the real-time NMR spectroscopy to study phosphorylation events of intrinsically disordered regions in vivo using the "unique domain" of c-Src in *Xenopus* oocytes combined with a number of phosphatases and kinases as a proof-of-concept.

In order to investigate the modulating factors for helix propensity in IDPs, Iesmantavicius et al.[66] employed selective mutation and observation by NMR spectroscopy. Using the protein activator for thyroid hormone and retinoid receptors as a model, they observed that long-range hydrophobic helix-helix interactions stabilize distant helices and that transient secondary and tertiary structure are often coupled.

Diana et al.[67] demonstrated a method for following protein-protein interactions in cell lysates using fast NMR spectroscopy, and applied this technique to the human prune protein, a phosphoesterase with an intrinsically disordered C-terminal region.

Theillet et al.[68] introduced an NMR protocol for site-specific mapping and time-resolved monitoring of protein phosphorylation reactions. Phosphorylation is quantified with consecutive 2D ^{1}H-^{15}N band-selective optimized-flip-angle short-transient (SOFAST)-heteronuclear multiple-quantum (HMQC) NMR experiments, a method which is particularly amenable to intrinsically disordered proteins and regions, and offers significant advantages over western blotting and mass spectrometry.

Kumar[69] developed a protocol for high-throughput protein backbone assignment using NMR with particular applicability to a range of IDPs. The method entails the use of a single reduced dimensionality experiment and achieves better dispersion through a linear combination of backbone $^{13}C^{\alpha}$ and $^{13}C'$ chemical shifts.

Single-molecule spectroscopy

Hofmann et al.[70] used single-molecule spectroscopy to investigate the combined effect of pH and denaturants on the dimensions of the intrinsically disordered cold shock protein. Interestingly, the authors found that this protein became more compact at low pH levels, despite an increase in net charge that should lead to the increased repulsion. The authors hypothesize that this is due to the complicated interplay of electrostatic interactions, hydrophobic effects, proton binding entropy, and denaturant binding.

In-cell analysis of IDPs

Gruet et al.[71] used directed random mutagenesis (DRM) and a protein complementation assay based on green fluorescent protein reconstitution to identify the molecular determinants of binding efficiency in IDPs. Using a randomly generated library of the intrinsically disordered region of the measles virus nucleoprotein along with its binding partner, these authors demonstrated the usefulness of this method by identifying a critical residue that was previously unidentified, as well as regulatory sites which dampen the interaction.

Miscellaneous

Gray et al.[72] introduced an assay that measures self-assembly and applied this assay to identify the biochemical determinants which regulate dimerization in the oncoprotein ARG2. Interestingly, the authors found that peptides derived from the intrinsically disordered N-terminal region helped to stabilize the resulting oligomers.

Jain et al.[73] discuss time-resolved small X-ray scattering (SAXS) in the study of protein dynamics. Using a newly developed 20-microchannel microfluidic continuous-flow mixer in the SAXS settings, the authors were able to demonstrate the unfolding dynamics of ubiquitin and suggested that this method might be a useful tool in the study of the functions of intrinsically disordered proteins, protein mis-folding, and protein aggregation.

Looking at IDPs/IDPRs in Diseases

IDPs in neurodegenerative diseases

α-Synuclein is a classic IDP[74,75] that is observed to form soluble oligomers and insoluble aggregates in various synucleinopathies such as Parkinson disease,[76-80] which is often triggered by exposure to various agrochemicals.[81] The α-synuclein-dopamine interaction was mentioned previously in this digest, as a proof of principle in combining MD trajectories and 2D NMR chemical shifts (see Methods of the IDP/IDPR analysis section). Illes-Toth et al.[82] further probed the α-synuclein-dopamine interaction using ESI-IMS-MS, and found that the binding stoichiometry is characterized by a 1:3 ratio. An extended conformation of α-synuclein was required for dopamine binding, and in fact dopamine causes the population of α-synuclein to favor extended conformations. This result was confirmed with tyrosine, which is structurally similar to dopamine.

A study by Esteban-Martin et al.[83] used various biophysical techniques on α-synuclein, and found that monomeric α-synuclein participates in transient tertiary interactions that are precursors to fibrillar β-strands. The authors suggest that as these interactions are found in the fibrillar state but not in the amyloid state, these transient tertiary interactions must undergo an additional step of redistribution for amyloid structures to occur.

Diabetes

Islet amyloid polypeptide (IAPP), which can contribute to type II diabetes, is an IDP that was briefly discussed in the last digest.[2] Wu and Shea[84] used MD simulations to compare amyloidogenic and non-amyloidogenic forms of IAPP. The authors find a common helix-coil structure essential to normal activity, and a unique β-hairpin only found in the amyloidogenic form. The authors suggest that this unique structural element is a potential contributor to amyloidogenesis, representing a potential target to suppress the amyloidogenicity of IAPP.

Viral IDPs

The hepaciviruses, including hepatitis C (HCV) and GB virus B (GBV-B) contain a long, intrinsically disordered protein called NS5A. Lauck et al.[85] reported evolutionary insights based on sequencing of a new hepacivirus, guereza hepacivirus (GHV), that clusters phylogenetically with GBV-B, and that was recently discovered in old world primates. The authors found that the NS5A protein in GHV had an unprecedented amount of intrinsic disorder, which suggests that the tolerance for structural variability in this protein is higher than previously established. This discovery also introduces an additional model for understanding these important viruses.

Src homology 3 (SH3) domains interact with many target proteins via a canonical PxxP motif. However, some proteins interact with SH3 domains through non-canonical motifs. In another study characterizing the function of NS5A, Schwarten et al.[86] examined the Bin1-SH3 domain interaction with NS5A. Using biophysical techniques, the authors demonstrate that NS5A has 1 canonical binding motif and 2 non-canonical motifs. Further investigation of these non-canonical motifs revealed that upon interaction with SH3, these motifs lose structure, representing an order-to-disorder transition characteristic of a fuzzy complex and therefore represent an illustrative example of the transient[87] or conditional, or dormant disorder phenomenon.[88,89]

Wang et al.[90] investigated the formation of double-membraned vesicles by poliovirus. They demonstrated that the poliovirus protein 3AB is sufficient to induce the formation of double-membraned liposomes. NMR reveals that the N-terminal region of 3AB is intrinsically disordered, and the authors propose that this region may stabilize intermediates crucial for the invagination process.

The HIV-1 protein Rev is an RNA binding protein essential for viral replication. Earlier computational analysis revealed that all HIV proteins contain some regions of intrinsic disorder, with disorder being unevenly distributed between these proteins.[91]

In fact, protease (PR), envelope proteins gp120 (SU), and gp41 (TM), as well as reverse transcriptase (RT or p51) and integrase (IN or p41) were predicted to be mostly ordered, whereas Rev, Tat, p6, and p6* were predicted to be mostly disordered. Noticeable disorder was also predicted in many auxiliary proteins, and in the structural proteins p17 (MA), p24 (CA), and p7 (NC).[91] In support of these earlier observations, Casu et al.[92] used multi-dimensional NMR, CD and MD simulations to observe the structural transitions of Rev and explore the conformational landscape of this IDP. The obtained results suggest that the arginine-rich RNA binding motif of Rev is intrinsically disordered and may transition via induced fit or coupled binding-folding. The authors propose that these findings might give a framework for a general strategy for arginine-rich RNA binding proteins.

Nicolau and Giuliatti[93] used molecular modeling to examine the structure of the E7 oncoprotein in HPV, a protein predicted to be intrinsically disordered in the N-terminal region.[94,95] The authors constructed models of the E7 protein from both low risk and high risk types of HPV and found that while trajectory analysis predicted greater instability in the high risk types, secondary structure could be produced in all types via simulation. ANCHOR prediction was used to identify several disorder-based interaction regions that may be potential drug targets.

preS1 is an intrinsically disordered hepatitis B virus surface-binding protein which binds to the human protein γ2-adaptin. Using NMR, X-ray crystallography and MD simulations, Jurgens et al.[96] explored the structural properties of this interaction to provide insights into this potential drug target. The authors found that preS1 was able to bind through host motif mimicry, and suggested that short linear motifs in disordered regions may provide a way through host defenses.

IDPs/IDPRs as Drugs or Drug Targets

The N-terminal domain (NTD) of the androgen receptor (AR) is a promising target for hormone therapies, because it is required for transcriptional activity. However, the inherent flexibility of this intrinsically disordered region makes it a challenging drug target. Myung et al.[97] described EPI-001, a small-molecule antagonist of the AR NTD, and found that it and its analogs bind covalently to the NTD. All of the analogs reduced growth of castration-resistant prostate cancer xenografts, demonstrating that this methodology of designing small-molecule IDP/IDPR antagonists could be particularly useful for specifically targeting IDP/IDPRs.

Zhu et al.[98] combined a fragment-based drug design approach with MD simulations to dock small molecules to the Aβ monomer, with the objective of stabilizing the monomer and preventing toxic oligomerization. Their approach identified several "hot spots" in the Aβ peptide (similar to Rosenmen et al.[48] covered in this digest in the Methods for IDP/IDPR Analysis section), representing a step toward targeting monomeric Aβ.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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