



## Targeting intrinsically disordered proteins in rational drug discovery

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# EXPERT OPINION

1. Introduction
2. IDPs in function and disease
3. Atomic level characterization of IDPs/IDPRs
4. Targeting disordered proteins for drug design
5. Conclusion
6. Expert opinion

## Targeting intrinsically disordered proteins in rational drug discovery

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**Introduction:** Intrinsically disordered proteins (IDPs) and intrinsically disordered protein regions (IDPRs) have gained wide recognition over the past decade due to their versatile roles in cell physiology and pathology. A large repertoire of IDPs/IDPRs has been implicated in numerous diseases, making them potential targets for therapeutic intervention. Recent advances in experimental methods and computational approaches have enabled detection and characterization of these highly dynamic proteins at atomistic detail, thus facilitating disorder/dynamic-based drug discovery.

**Areas covered:** This article presents an overview of the functional relevance and pathological implications of IDPs/IDPRs in cells. The authors outline the currently available experimental methods employed for structural characterization of these proteins. They also exemplify the practical limitations encountered during such characterization and ways to overcome them. Taken together, the article discusses the plausibility of exploiting protein disorder for drug targeting.

**Expert opinion:** Disorder-based drug targeting is gearing up in the realm of novel drug discovery approaches. Tools for probing the molecular features of IDPs and IDPRs are rapidly improving and start to provide accurate descriptions of the complex ensembles populated by IDPs/IDPRs. They thus pave the way for the development of drug molecules, which specifically target disease-associated disorder.

**Keywords:** drug discovery, intrinsically disordered protein, neurodegeneration, NMR spectroscopy

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### 1. Introduction

#### 1.1 Discovering the disorder

The central dogma postulates that the biological information is relayed precisely in unidirectional manner from the nucleotide sequence (DNA/RNA) to the protein. The ultimate functionality of a gene is encrypted in the ordered, three-dimensional (3D) structure of the encoded protein.[1] The hierarchy of protein folding follows a step-wise formation of secondary, tertiary and quaternary contacts within the nascent polypeptide chain, eventually forming a well-defined 3D structure. While this structure–function paradigm holds true for a majority of the proteins structurally characterized thus far, the discovery of extensive disorder in proteins has critically altered this general perception. The advent of whole-genome sequencing and proteomic analysis complemented with computational approaches has unraveled the presence of disordered and highly dynamic, yet, functionally active proteins along the evolutionary chain.[2–5] These proteins, termed ‘intrinsically disordered proteins’ (IDPs), do not fold spontaneously into a compact 3D architecture under physiological conditions, instead, exist as dynamic ensemble of rapidly inter-converting states.[6–11] In addition, rigidly folded globular proteins are often interspersed with long stretches of disordered regions, so-called

**Article highlights**

- Intrinsically disordered proteins (IDPs) are implicated in numerous diseases and serve as therapeutic targets.
- Recent advances in experimental and computational approaches have enabled detection and characterization of these highly dynamic proteins at atomistic detail.
- NMR spectroscopy is a key technology for characterization of IDPs.
- Because IDPs fundamentally exist as conformational ensembles, drug design approaches against IDPs should take into account their dynamic nature.
- Targeting IDPs in complex with key physiological and pathological interaction partners is a largely unexplored area.

This box summarizes key points contained in the article.

‘intrinsically disordered protein regions’ (IDPRs). IDPRs assist in tethering different protein domains by acting as linkers or directly contribute to the functional role of the protein.[8,12]

Based on the overall structural content, proteins can be broadly classified into four groups: random coil, pre-molten globule, molten globule and stably folded. Within the cell milieu, a given protein can perform excursions between these states, depending on the cellular requirement or for execution of function.[13] IDPs predominantly populate disordered states, but may attain a folded state, either locally or globally upon binding to an appropriate target.[8,14–16] In addition, folded globular proteins can also sample more disordered states, which are weakly populated under native conditions but exist in equilibrium with the folded conformation.

## 1.2 Ubiquitous existence of IDPs

The post-genomic era has witnessed an explosive number of sequences, which encode functional proteins that lack structural homology with known folded proteins.[2,14,17–21] It is now estimated that intrinsic disorder is abundant in eukaryotic genomes with ~30% of eukaryotic proteins consisting of disordered regions extending over 50 or more contiguous residues.[18,22] In addition, the overall disorder content linearly increases from archaea to bacteria and eukaryotes,

perhaps to facilitate specific complex cell requirements in higher organisms.[19,23] Today several computational methods are available, which allow prediction of disorder on the basis of the amino acid sequence.[4,17,18,24] These prediction algorithms are based on sequence attributes such as hydropathy, distribution of polar residues and sequence complexity. A comprehensive compilation of structural and functional information of completely and partially disordered proteins can be obtained from Disprot, a curated database of experimentally verified disordered regions,[25,26] as well as from the protein ensemble database pE-DB.[27]

Besides contributing to cellular functions, IDPs and IDPRs can have adverse effects, which might be detrimental to cell viability or manifest as disease. In addition, factors such as defective splicing of exons, chromosomal translocation, point mutations and post-translational modifications can drive folded proteins into kinetically trapped disordered state.[21,28–33]

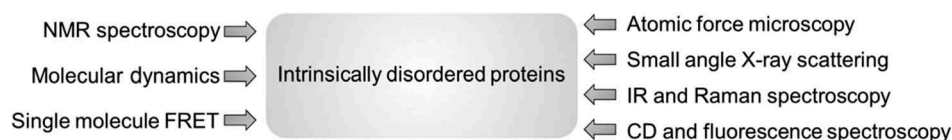
## 1.3 Scope of the review

Characterizing protein disorder and dynamics in the context of their biophysical and structural topographies, mechanistic details of their manifold roles in cellular processes and in their pathological manifestation in cells has greatly enhanced the current understanding of protein–function–disease relationships. This review provides insight into the multifaceted roles of IDPs in biological function and human disease, the challenges involved in characterization of these highly dynamic proteins, experimental tools for assessing their molecular features at high resolution (Figure 1) and plausible strategies to utilize disordered and dynamic proteins as specific drug targets. Several archetypal illustrations, though not exhaustive, are presented.

## 2. IDPs in function and disease

### 2.1 Disorder for functional multiplicity

Early reports on disorder-associated cellular functions have been projected as exceptions to the traditional ‘structure dictates function’ viewpoint. However, with the steady increase in the number of IDP and IDPR discoveries across all taxa, the importance of disorder for extending the



**Figure 1. Tools for analysis of the dynamic conformation of IDPs.** Atomic level descriptions of disordered protein ensembles are facilitated by quantitative observables from NMR spectroscopy and single molecule fluorescence techniques, which can be combined with computational methods (left panel). Overall molecular features, biophysical properties and morphologies of IDPs and IDP aggregates can be obtained from the experimental methods shown to the right.

spectrum of protein functions has been widely accepted. Several critical functions such as signaling, transcription, apoptotic regulation, enzyme activity and DNA/RNA binding are attributed to IDPs and IDPRs.[5,8,20,34–36]. In addition, some chaperones whose primary activity is to assist in proper folding of proteins also possess intrinsically disordered regions, which serve as auxiliary domains for binding misfolded proteins.[37,38]

The function of IDPs is modulated by a variety of post-translational modifications ranging from phosphorylation to acetylation, methylation, glycosylation and ADP-ribosylation.[5,20,34] Furthermore, a delicate equilibrium between monomeric, dimeric and higher-order oligomeric states can dictate the function and malfunction of IDPs.[39–41] Extended disordered regions in multi-domain proteins further act as flexible linkers between individual domains [5,12] and may contain regulatory sites.[16,36,42]

## 2.2 Protein disorder in disease

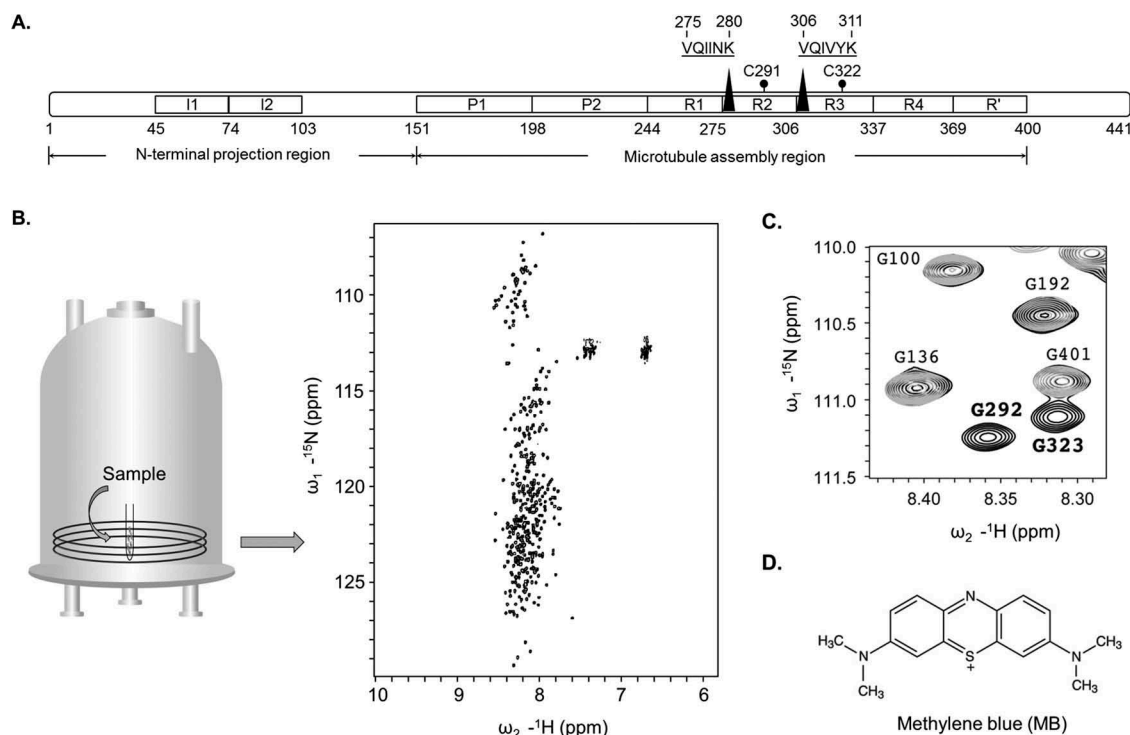
Apart from actively contributing to physiological roles in cells, IDPs and IDPRs critically contribute to the pathological manifestation of an array of maladies.[21,25,34,43] Statistical analyses of the involvement of disordered versus ordered proteins in human diseases have suggested that a significant proportion of diseases is connected to disordered segments of proteins extending from short residue stretches to entire polypeptide sequences.[44,45] Besides IDPs and IDPRs, protein folding based disorder may arise due to failed chaperone functions leading to an accumulation of misfolded proteins,[46] dysregulation of signaling cascades and uncontrolled protein modifications, as well as genetic variations and alternate splicing.[21,31,44,45,47,48] These are extensively discussed elsewhere and not within the scope of this review.

The overabundance of intrinsic disorder in human diseases has given rise to the ‘D<sup>2</sup>’ concept (disorder in disease).[25] IDPs/IDPRs were shown to have detrimental roles at various levels, with pathological effects ranging from inactivation of function to cell death.[21,34,45,48] In particular, misfolding and aggregation of IDPs is tightly connected to neurodegeneration and related disorders.[43] Misfolding and aggregation of IDPs results in a new toxic function through a so-called gain-of-toxic-function mechanism. In case of Alzheimer’s and Parkinson’s disease, the IDPs amyloid- $\beta$  (A $\beta$ ),  $\alpha$ -synuclein and Tau protein are of prime interest, because aggregates of these proteins are found as insoluble deposits in the brain of patients and mutations in the proteins lead to early disease-onset. In addition, the importance of IDPs in cancer is increasingly recognized. For example, the tumor suppressor protein, p53 is – due to its large signaling hub activity and its binding multiplicity – a widely studied protein with large disordered regions.[49–51] cMyc–Max interaction is yet another cancer associated pair, which has been investigated in great detail and its structural features have been comprehensively reviewed.[52–55] Likewise, the IDP  $\alpha$ -Fetoprotein

is considered as bio-marker for abnormal fetal development and cancers.[56] Extending the connection of IDPs with disease, amylin (or islet amyloid polypeptide) is important for insulin-independent diabetes.[57] Also, intrinsic disorder has been shown to be prevalent in proteins associated with cardiovascular diseases.[58]

Considering the growing interest in neurodegeneration, the IDPs Tau and  $\alpha$ -synuclein are discussed in the following in more detail. The microtubule-associated protein Tau is a neuronal protein, which is predominantly expressed in the central and peripheral nervous system. Both experimental studies and computational prediction algorithms show that even the longest Tau isoform with 441-residue is completely disordered. The protein contains an N-terminal projection domain, which is believed to be important for the spacing of microtubules,[59,60] while the C-terminal part of the protein contains several pseudo-repeat sequences, which bind to microtubules (Figure 2A). Upon binding to the interface between tubulin heterodimers, short residue stretches in the repeat domain of Tau fold into a defined conformation.[61,62] Microtubule-binding and misfolding is extensively modulated by post-translational modifications including phosphorylation, ubiquitination and acetylation.[63–66] Under pathological conditions, hyperphosphorylation of Tau is triggered by a cascade of kinases, displacing Tau from microtubules and/or affecting microtubule-assembly. Although impaired Tau function might be compensated at least partially by other microtubule-associated proteins, hyperphosphorylated Tau proceeds to form oligomers and subsequently neurofibrillary tangles, which are a pathological hallmark in the brain of Alzheimer’s disease patients.[67–69] Tau-related neurodegenerative disorders, so-called tauopathies, also include Pick’s disease and progressive supranuclear palsy.[70] Furthermore, Tau has been associated with Parkinson’s disease in genome-wide association studies.[70,71] A variety of strategies have been proposed to interfere with Tau-mediated neurotoxicity ranging from the regulation of kinases and phosphatases to modulation of Tau-aggregation and stabilization of microtubules by anti-cancer drugs.[72–75]

The 140-residue IDP  $\alpha$ -synuclein is important for synaptic vesicle trafficking, wherein the protein attains a helical conformation upon binding to vesicles.[76,77] However,  $\alpha$ -synuclein is particularly well known, because aggregates formed by this protein in the brain are implicated in Parkinson’s disease and numerous synucleinopathies.[78]  $\alpha$ -Synuclein aggregates are found in different brain regions, causing an overlap of symptoms and making the prognosis problematic. In addition,  $\alpha$ -synuclein aggregates are found in distinct morphologies, classified as Lewy bodies, Lewy neurites (dystrophic neurites), glial cytoplasmic inclusions, neuronal cytoplasmic inclusions and axonal spheroids.[78] Continued efforts are being made to assign these disorders to a defined misfolded state of the protein. Taken together, each IDP is in itself a complicated system demanding a robust strategy for pathophysiological interpretation.



**Figure 2. Targeting the Tau protein by small molecules.** **A.** Schematic representation of the longest Tau isoform, 2N4R. 2N implies the number of inserts (I1 and I2) in the N-terminal projection region and 4R indicates the number of repeat regions in the C-terminal half of the protein. The microtubule assembly domain spans the proline-rich regions (P1 and P2), the pseudo-repeat sequences (R1–R4) and a region R', which is partially similar to R1–R4. The hexapeptide stretches VQIINK and VQIVYK (marked in triangles) are essential for aggregation of Tau into paired helical filaments. **B.** Representative 2D-[ $^1\text{H}$ - $^{15}\text{N}$ ]-HSQC spectrum of 441-residue Tau protein. Small amide proton chemical shift dispersion is indicative of structural disorder. **C.** Superposition of a selected region of a 2D-[ $^1\text{H}$ - $^{15}\text{N}$ ]-HSQC spectrum of Tau in presence (grey) and absence (black) of methylene blue. Methylene blue specifically modifies the cysteine residues (C291 and C322, highlighted in panel A) in Tau monomers, resulting in broadening of adjacent glycine peaks (G292 and G323).[130] **D.** Molecular structure of the Tau aggregation inhibitor methylene blue. Methylene blue stabilizes an aggregation incompetent monomer and prevents the formation of filaments and toxic oligomeric precursors.

### 3. Atomic level characterization of IDPs/IDPRs

#### 3.1 Experimental tools for IDP characterization

Because IDPs are involved in a wide range of life-threatening disorders, there is great interest in understanding their molecular features at microscopic detail. IDPs when in solution are best described as ensembles of heterogeneous, rapidly inter-converting states, which transiently populate elements of secondary structure and tertiary contacts without folding into a rigid, globular structure.[6–11] Because of their highly dynamic nature and the low affinity of many of the interactions, which IDPs are involved in, X-ray crystallography and single-particle electron microscopy can only provide limited structural information. For example, gross morphologies of oligomers and aggregates can be imaged by cryo-electron microscopy, transmission electron microscopy and atomic force microscopy (Figure 1).[79,80] Light scattering is another approach sensitive to aggregates and is used to assess

the polydispersity of protein aggregates in solution. In addition, a variety of biophysical tools such as circular dichroism, Raman spectroscopy and small-angle X-ray scattering can provide insight into the basic molecular properties of IDPs. NMR spectroscopy, small-angle X-ray scattering and single-molecule fluorescence techniques are particularly useful because they provide complementary information and together allow access to an atomistic picture as well as the conformational landscapes of IDPs/IDPRs (Figure 1). In particular, NMR spectroscopy provides residue-specific information, while small-angle X-ray scattering allows insight into the overall shape of ensembles and single-molecule fluorescence measurements avoid ensemble averaging.[6,81–95]

NMR spectroscopy (Figure 2B) is a versatile technique for the analysis of the dynamic conformations of IDPs/IDPRs. A wide range of residue- and atom-specific NMR parameters can be measured that are sensitive for protein dynamics from pico-to-microseconds as well as secondary structure and



tertiary contacts. Particularly powerful are two-dimensional  $^1\text{H}$ - $^{15}\text{N}$  correlation spectra, which provides the footprint of a given protein. Well folded proteins display good spectral dispersion in these spectra, while IDPs/IDPRs yield poor dispersion in the proton dimension resulting in spectral crowding (Figure 2C). Key to a detailed structural and dynamic analysis of IDPs/IDPRs and their interaction with binding partners, is the one-to-one mapping of NMR cross peaks to specific protein nuclei. To this end, a variety of NMR experiments are nowadays available.[96–99] Once this so-called assignment process is complete, NMR chemical shifts serve as excellent indicators of secondary structural preferences. Deviations from expected random coil chemical shifts can be used to assess the propensities for  $\alpha$ -helix,  $\beta$ -strand, and PPII helix within stretches of residues.[100] Other useful NMR parameters include residual dipolar couplings and Nuclear Overhauser Enhancement contacts, [101–104] although the later mostly report on local structure, because tertiary structure is not sufficiently stable in IDPs/IDPRs. A particularly useful method for probing the dynamic ensemble of IDPs and their interaction with binding partners is paramagnetic relaxation enhancement. [105–107] Access to paramagnetic relaxation enhancement requires the presence of a paramagnetic center in the IDP, which is usually achieved through attachment of a paramagnetic spin label to a native or engineered cysteine residue. Paramagnetic relaxation enhancement allows the analysis of long-range contacts even when they occur only transiently. Similar to the fluorescence-based technique of Förster resonance energy transfer, in this case paramagnetic tag is attached to the IDP, which then perturbs the NMR signals in a distance-dependent manner. Because all nuclei represent “reporters”, this NMR method simultaneously probes a very large number of inter-residue contacts. For example, the molecular ensembles populated by the proteins Tau and  $\alpha$ -synuclein in solution were analyzed by a combination of chemical shifts, scalar couplings, residual dipolar couplings and inter-residue distance information derived from paramagnetic relaxation.[106–109] Conformational dynamics plays a pivotal role in influencing all of the above mentioned measurements. In addition, molecular motions can be directly probed by spin relaxation measurements over a wide range of time scales.[110,111]

### 3.2 Computational approaches

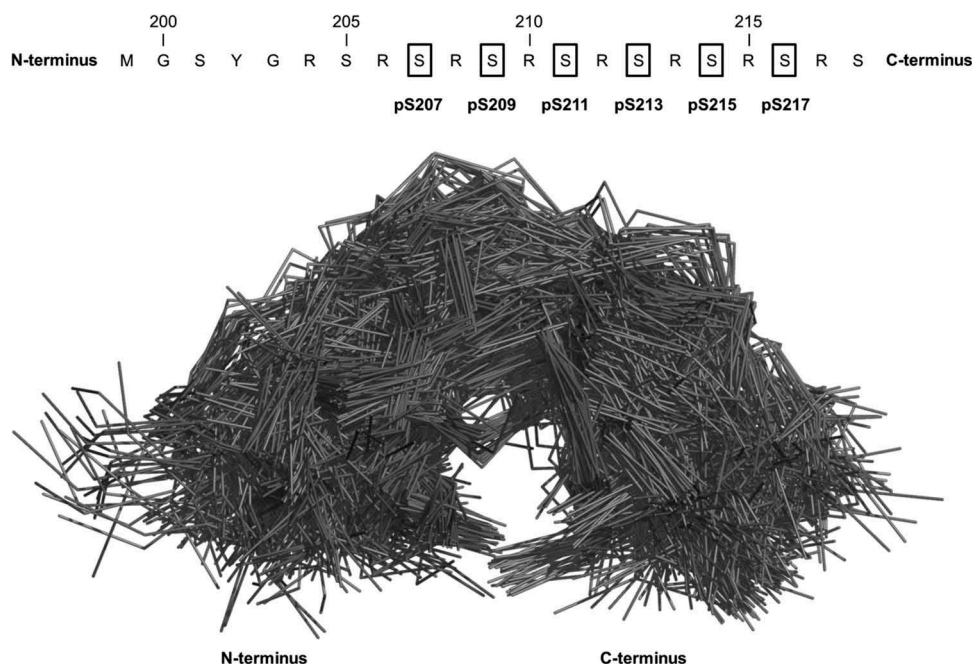
Because of the inherently large number of degrees of conformational freedom, however, ensembles of IDPs remain underdetermined.[7,9,101,112,113] In addition, molecular dynamics simulations of IDPs require very long simulation time scales, in order to converge and are influenced by the choice of force field.[114,115] Therefore, sample-and-select approaches have been developed, whereby sub-ensembles are derived on the basis of experimental NMR and small-angle X-ray scattering data from a broader distribution of

molecular conformations.[7,9,101,112,113] For example, conformational ensembles were selected on the basis of a large set of experimental NMR data from a molecular dynamics (MD) trajectory of the arginine-serine rich region of the serine/arginine-rich splicing factor (SRSF1). The conformational ensembles revealed that phosphorylation of the serine residues results in a dynamic switch in SRSF1 [113] (Figure 3). In an alternative approach, NMR parameters were directly enforced in molecular ensemble calculations of phosphorylated and non-phosphorylated Tau fragments. The analysis showed that phosphorylation of Tau at threonine 231 results in an intramolecular salt-bridge, which can compete with intermolecular salt-bridges to tubulin.[104] In yet another study, conformational propensities of a  $\beta\gamma$ -crystallin, hahellin, were determined by the combined use of NMR spectroscopy and replica exchange MD simulations.[116] MD simulations are also expected to play a significant role in the modeling of IDP ensembles based on statistical thermodynamics. In addition to aiding in structure calculations, information about protein dynamics can also be assessed by computational means. In this endeavor, the HYCUD approach, hydrodynamic coupling of domains, was developed, which predicts the effective rotational correlational time in multi-domain proteins and large IDPs.[117,118] This information might be used to detect disorder-to-order transitions in dynamic biomolecules.

## 4. Targeting disordered proteins for drug design

The widespread implication of IDPs in disease suggests that IDPs are viable targets for therapeutic interventions. [55,119,120] Indeed, the role of IDPs as crucial hubs in protein interaction networks and their large binding multiplicities, offer an exciting platform for specifically targeting disordered segments. Different approaches can then be followed to target IDPs including the development of small molecules that either: (i) directly bind to the disordered ensemble, or (ii) that bind to a binding partner and inhibit IDP binding or stabilize its bound state, and (iii) that affect a modification pathway (a kinase, for example). For strategies (ii) and (iii) conventional drug design approaches can be followed, which utilize 3D structural information of the target protein for identification of druggable cavities. In addition, computational drug discovery approaches facilitate the prediction of druggability and effect of small molecules on IDP interactions.[121–125] In contrast, the dynamic nature of IDPs severely hampers conventional drug design approaches based on static 3D structures and thus complicates the direct targeting of IDP ensembles.

Currently existing drug design strategies for IDPs include the development of small molecule and peptide inhibitors, [21,72,126,127] arresting order-to-disorder transitions,[126] targeting regulatory elements [21,34] and the modulation of post-translational modifications.[20] For example, hyperphosphorylation of Tau is a pathological hallmark of Alzheimer's



**Figure 3. Ensemble of conformations populated by the serine/arginine-rich region of SRSF1 in its phosphorylated state.** SRSF1 is a classical serine/arginine (SR) protein, which plays a crucial role in RNA metabolism. Combination of NMR spectroscopy and MD simulations indicated a phosphorylation-induced conformational switch of the RS dipeptide repeat sequence from a highly disordered state to a partially rigid, arch-like structure [113] (as shown in the figure). Phosphorylated serine residues are highlighted in the amino acid sequence.

disease and cellular levels of phosphorylated Tau are regulated by a cascade of kinases. An important kinase is glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ). Several small molecule drugs have been proposed to inhibit the activity of GSK3 $\beta$  and thus reduce the level of phosphorylated Tau in neuronal cells.[73]

#### 4.1 Targeting IDPs important for neurodegenerative diseases

Because several biomolecules, which play a key role in Alzheimer's, Parkinson's and other neurodegenerative diseases, are IDPs, there is great interest in developing therapeutic strategies, which directly target the dynamic structure of these molecules (strategy (i) described above). For example, the tricyclic phenothiazine methylene blue, which has a history of diverse medical applications including the use for distinct cellular targets,[128] has been shown to have promising effects in preventing Tau aggregation and reached phase 3 clinical trials in human Alzheimer's disease patients.[129] A mechanistic analysis revealed that methylene blue and its derivatives inhibit Tau aggregation by the interplay of reduction/oxidation of Tau's two native cysteine residues.[130] Specific modifications of these residues retain Tau in its monomeric state, thereby inhibiting the formation of toxic oligomeric and fibrillar aggregates [130] (Figure 2C and 2D).

The aggregation inhibitor phthalocyanine tetrasulphonate (PcTS), on the other hand uses a different mechanism. The porphyrin PcTS interferes with Tau filament formation by binding to specific aromatic residues, thereby directing the protein into soluble non-toxic oligomers.[72] These studies show that it is not sufficient to analyze only the oligomerization state of IDPs in order to design new therapeutic strategies, but a crucial step is to elucidate their structure and connect structure to toxic properties.

IDPs fundamentally exist as conformational ensembles. The design of small molecules directed against IDPs should therefore be based on conformational ensembles, which best describe the dynamic nature of IDPs. Indeed, the drug-like compound phenyl sulfonamide, ELN484228, has been proposed as a small molecule, which targets the disordered soluble state of  $\alpha$ -synuclein. Using *in silico* structure-based computational docking, several small molecules were identified, which access binding pockets in the molecular ensemble of  $\alpha$ -synuclein. Of these, ELN484228 was shown to have significant cellular activity and to restore disrupted synaptic vesicle trafficking.[131]

When devising strategies to target the structure of IDPs, it is important to understand the distinct conformations IDPs can populate in different environments. An important example is the 140-residue protein  $\alpha$ -synuclein, which is highly dynamic in solution and a prototypical IDP.[132]  $\alpha$ -Synuclein folds into a

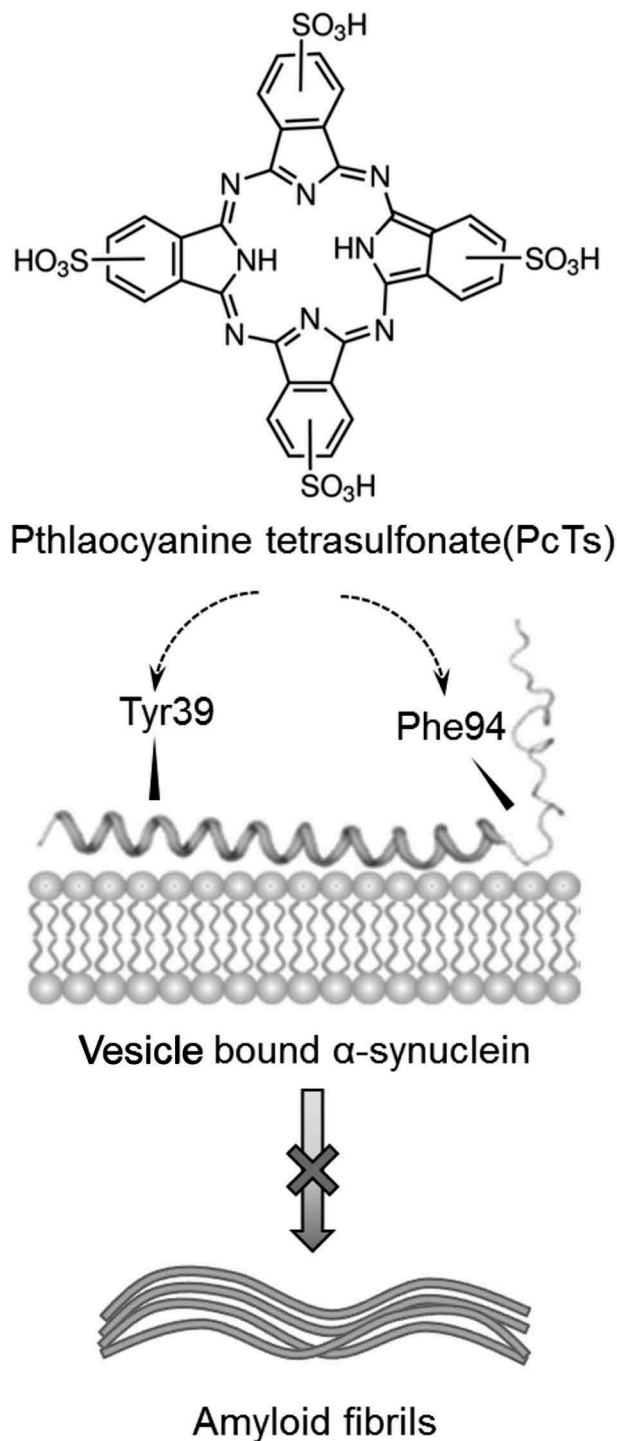
rigid helical structure when it binds to vesicles.[76,77] Thus, small molecules might be developed, which specifically bind to the helical, vesicle-bound structure of  $\alpha$ -synuclein, thereby averting its misfolding and neurotoxic aggregation. From a series of NMR-based and complementary biophysical approaches, it was shown that such an approach might indeed be viable: PcTS, but not the Parkinson's disease drug selegiline, directly bound to residues Tyr39 and Phe94 of vesicle-bound  $\alpha$ -synuclein and thereby stabilized its helical state [126] (Figure 4). Stabilization of the helical structure delays aggregation of  $\alpha$ -synuclein and thus can modulate its neurotoxic effects.

Apart from small molecule inhibitors, molecular tweezers, which are essentially noncyclic molecules, can bind host proteins through non-covalent interactions such as hydrogen bonding, pi-pi stacking and electrostatic interactions. For example, the lysine specific molecular tweezer CLR01 was shown to disintegrate preformed fibrils of  $\alpha$ -synuclein and prevent the formation of oligomers. *In vivo* assays and model studies on Zebra fish embryos have also suggested that CLR01 suppresses  $\alpha$ -synuclein induced apoptosis and toxicity, thereby enhancing phenotypic survival.[133, 134]

While the above discussed strategies directly interfere with the causal IDPs and are aimed at arresting the formation of toxic oligomers and/or fibrillization, anti-amyloid therapies guided against A $\beta$ 40 and A $\beta$ 42 oligomers currently employ multiple approaches, which include modulation of enzymes that regulate A $\beta$  production, clearance of the A $\beta$  pool, manipulation of the A $\beta$ 40:A $\beta$ 42 ratio and increasing the neuronal resistance to toxic A $\beta$ . Though several of the proposed molecules proved effective in alleviating neurotoxicity, the success has been limited due to factors such as non-specific targeting, failures in restoration of cognition and inability to surpass the blood brain barrier.[135]

#### 4.2 Modulation of IDP interactions in cancer

IDP-based rational drug discovery also finds applications in cancers. A crucial target is the protein p53 and its interaction with MDM2. For example, nutlins are cis-imidazoline analogs, which were widely used for targeting p53-MDM2 interactions.[55,136] A comprehensive list of small molecule MDM2 antagonists can be found in.[137] Because these small molecules do not directly bind to the disordered regions of p53, but block the binding site on MDM2, they can be classified as modulators of IDP interactions. Heterodimeric cMyc-Max is another potential target because of its role in numerous cancers.[21] cMyc is a helix-loop-helix leucine zipper that is intrinsically disordered. Upon heterodimerization with its binding partner, Max, the cMyc-Max complex assumes a coiled coil structure, which recognizes the E-box sequence during gene regulation.[52] In several cancers, leukemias and lymphomas, cMyc is deregulated, triggering its oncogenic function. Direct or indirect inhibition of cMyc might therefore be a



**Figure 4. Inhibition of the pathogenic aggregation of vesicle bound  $\alpha$ -synuclein by PcTS.** The N-terminal ~100 residues of  $\alpha$ -synuclein adopt an  $\alpha$ -helical conformation upon binding to vesicles. The small molecule aggregation inhibitor PcTS binds to the aromatic residues Tyr39 and Phe94 of  $\alpha$ -synuclein and thereby stabilizes the  $\alpha$ -helical conformation. [126] This delays pathogenic aggregation of  $\alpha$ -synuclein in a physiological vesicle environment.



viable therapeutic strategy for developing anti-cancer drugs. A library of cMyc-Max dimer inhibitors were proposed, which had varying effects on dimerization of the complex. Most of these inhibitors directly influence the DNA binding of the Myc-Max dimer.[21,120,138] Recently, a novel approach to disrupt the coiled-coil conformation of the Myc-Max complex has been proposed. In this approach, the  $\alpha$ -helix mimetic, JKY-2-169, specifically recognizes and disrupts the helical interface of the heterodimer.[127] Another interesting class of disordered proteins is the BCL-2 family of proteins that control the mitochondrial pathway of apoptosis. BH3-only protein act as antagonists of these prosurvival members and can selectively induce apoptosis in malignant cells. Novel therapeutic approaches for cancer therapy use BH3-mimetics to directly target these proapoptotic proteins.[139–141]

## 5. Conclusion

Intrinsically disordered proteins and disordered regions in proteins display remarkable heterogeneity in terms of structural architectures, binding multiplicity, functional diversity and regulation modes. Furthermore, IDPs play a crucial role in several neurodegenerative diseases, as well as in cancers, diabetes and cardiovascular diseases. Dedicated experimental and computational platforms are focused on rapid identification and delineating the molecular details of IDPs. Powerful tools are now available for determination of conformational ensembles of IDPs in their free form and bound to physiological binding partners, for analysis of the kinetics and thermodynamics of IDP aggregation, for cell-based assessment of IDP toxicity as well as transgenic animal models to replicate the diseased state.

## 6. Expert opinion

Intrinsically disordered proteins have versatile roles in cell physiology and a large repertoire of IDPs has been implicated in different diseases. This makes IDPs potential targets for therapeutic intervention. Because IDPs fundamentally exist as conformational ensembles, drug design approaches against IDPs should take into account their dynamic nature. The dynamic nature of IDPs favors low binding affinity without losing specificity. At the same time, other IDPs may co-exist, which can bind to the same drug molecule. Such non-specific targeting can have adverse effects on healthy tissues and lead to detrimental side effects. Although this is a common concern also for well-folded proteins, the likelihood is more profound in case of IDPs, because of the higher plasticity of IDPs.

Tools for probing the molecular features of disordered proteins are rapidly improving and starting to provide accurate descriptions of their conformational ensembles. It is likely that at the core of these endeavors NMR spectroscopy will remain and further grow, because of the exquisite

versatility and sensitivity of NMR spectroscopy for conformational changes and dynamics at atomic resolution. Although a large variety of NMR experiments have been developed, which are specifically optimized for disordered proteins, there is still an urgent need for further improvements in sensitivity and resolution. Additionally, NMR-based analysis of IDPs in combination with other biophysical tools should be streamlined and better integrated, in order to allow a large user community easy access to this important technology. Along these lines, the setup of a pipeline can be imagined, which integrates data acquisition, automatic processing and peak picking, followed by automatic resonance assignment and ensemble determination, such that non-NMR experts might be able to perform an NMR-based analysis of IDPs in a similar manner as is currently done for folded proteins using circular dichroism. In parallel, improvements in MD simulations are required with force fields optimized for IDPs, such that the simulations accurately reproduce experimental data.

Direct targeting the conformational plasticity of IDPs is still in the very beginning. With a better understanding of the atomic details of the conformations of IDPs, small molecules might be developed which stabilize distinct IDP conformations. Stabilization of subsets of conformations of a particular IDP might provide the means to modulate IDP-protein interactions and IDP misfolding. Further insight is also expected once experimentally verified conformational ensembles of IDPs in complex with small molecules become available. A particularly promising area for drug design might be molecules, which are specifically designed to target the conformation of IDPs in complex with key physiological and pathological interaction partners. In case of  $\alpha$ -synuclein-mediated neurotoxicity this might be the development of small molecules, which specifically bind to the helical structure of vesicle-associated  $\alpha$ -synuclein and efficiently pass the blood–brain barrier. Alternatively, small molecules might be developed, which modulate the interaction of  $\alpha$ -synuclein and Tau with chaperone proteins, such as Hsp70 and Hsp90. Consistent with this hypothesis, screening of small molecules for IDP-mediated diseases should not be limited to the soluble, disordered state, but be extended to the complex of IDPs with crucial interaction partners. Besides directly modulating the structural properties of IDPs, a variety of indirect approaches can be imagined. In these approaches, the aim would be to influence the activity of key regulators that dictate the function of IDPs. This might include the translocation of pathogenic aggregates into the proteasome complex or mobilization of membrane-bound aggregates for degradation.

In summary, IDPs provide a unique opportunity for novel drug development approaches.

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## Declaration of interest

M Zweckstetter and S Ambadipudi are employees of German Center for Neurodegenerative Diseases (DZNE) who support this manuscript. M Zweckstetter is also an employee of the

Max Planck Institute for Biophysical Chemistry. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

## Bibliography

- Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers
1. Crick F. Central dogma of molecular biology. *Nature*. 1970;227(5258):561–563.
  2. Wright PE, Dyson HJ. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J Mol Biol*. 1999;293(2):321–331.
  3. Dunker AK, Obradovic Z. The protein trinity—linking function and disorder. *Nat Biotechnol*. 2001;19(9):805–806.
  4. Xue B, Dunbrack RL, Williams RW, et al. PONDR-FIT: a meta-predictor of intrinsically disordered amino acids. *Biochim Biophys Acta*. 2010;1804(4):996–1010.
  5. Wright PE, Dyson HJ. Intrinsically disordered proteins in cellular signalling and regulation. *Nat Rev Mol Cell Bio*. 2015;16(1):18–29.
  - **A comprehensive overview of multifunctional roles of IDPs.**
  6. Rezaei-Ghaleh N, Blackledge M, Zweckstetter M. Intrinsically disordered proteins: from sequence and conformational properties toward drug discovery. *Chembiochem*. 2012;13(7):930–950.
  - **Detailed review on experimental and computational methods for IDP characterization.**
  7. Jensen MR, Zweckstetter M, Huang JR, et al. Exploring free-energy landscapes of intrinsically disordered proteins at atomic resolution using NMR spectroscopy. *Chem Rev*. 2014;114(13):6632–6660.
  - **Comprehensive review on NMR and experimental techniques for IDP characterization.**
  8. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Bio*. 2005;6(3):197–208.
  9. Fisher CK, Stultz CM. Constructing ensembles for intrinsically disordered proteins. *Curr Opin Struc Biol*. 2011;21(3):426–431.
  10. Marsh JA, Teichmann SA, Forman-Kay JD. Probing the diverse landscape of protein flexibility and binding. *Curr Opin Struc Biol*. 2012;22(5):643–650.
  11. Mao AH, Lyle N, Pappu RV. Describing sequence-ensemble relationships for intrinsically disordered proteins. *Biochem J*. 2013;449(2):307–318.
  12. Dunker AK, Brown CJ, Lawson JD, et al. Intrinsic disorder and protein function. *Biochemistry*. 2002;41(21):6573–6582.
  13. Uversky VN. Natively unfolded proteins: a point where biology waits for physics. *Prot Sci*. 2002;11(4):739–756.
  14. Uversky VN, Dunker AK. Understanding protein non-folding. *Biochim Biophys Acta*. 2010;1804(6):1231–1264.
  15. Kovacs D, Szabo B, Pancsa R, et al. Intrinsically disordered proteins undergo and assist folding transitions in the proteome. *Arch Biochem Biophys*. 2013;531(1–2):80–89.
  16. Bah A, Vernon RM, Siddiqui Z, et al. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. *Nature*. 2015;519(7541):106–109.
  - **Atomic level study of the phosphorylation induced folding of an IDP.**
  17. Linding R, Jensen LJ, Diella F, et al. Protein disorder prediction: implications for structural proteomics. *Structure*. 2003;11(11):1453–1459.
  18. Ward JJ, Sodhi JS, McGuffin LJ, et al. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J Mol Biol*. 2004;337(3):635–645.
  19. Pancsa R, Tompa P. Structural disorder in eukaryotes. *PloS One*. 2012;7(4):e34687.
  20. Theillet FX, Binolfi A, Frembgen-Kesner T, et al. Physicochemical properties of cells and their effects on intrinsically disordered proteins (IDPs). *Chem Rev*. 2014;114(13):6661–6714.
  21. Uversky VN, Dave V, Iakoucheva LM, et al. Pathological unfoldomics of uncontrolled chaos: intrinsically disordered proteins and human diseases. *Chem Rev*. 2014;114(13):6844–6879.
  - **Systematic review on IDPs and their connection to disease.**
  22. Oldfield CJ, Cheng Y, Cortese MS, et al. Comparing and combining predictors of mostly disordered proteins. *Biochemistry*. 2005;44(6):1989–2000.
  23. Lobanov MY, Galzitskaya OV. How common is disorder? Occurrence of disordered residues in four domains of life. *Int J Mol Sci*. 2015;16(8):19490–19507.
  24. Prilusky J, Felder CE, Zeev-Ben-Mordehai T, et al. FoldIndex: a simple tool to predict whether a given protein sequence is intrinsically unfolded. *Bioinformatics*. 2005;21(16):3435–3438.
  25. Uversky VN, Oldfield CJ, Dunker AK. Intrinsically disordered proteins in human diseases: introducing the D2 concept. *Annu Rev Biophys*. 2008;37:215–246.
  26. Sickmeier M, Hamilton JA, LeGall T, et al. DisProt: the database of disordered proteins. *Nucleic Acids Res*. 2007;35(Database issue):D786–93.
  27. Varadi M, Kosol S, Lebrun P, et al. pE-DB: a database of structural ensembles of intrinsically disordered and of unfolded proteins. *Nucleic Acids Res*. 2014;42(Database issue):D326–35.
  28. Goedert M, Spillantini MG. A century of Alzheimer's disease. *Science*. 2006;314(5800):777–781.
  29. Romero PR, Zaidi S, Fang YY, et al. Alternative splicing in concert with protein intrinsic disorder enables increased functional diversity in multicellular organisms. *P Natl Acad Sci USA*. 2006;103(22):8390–8395.
  30. Hegyi H, Buday L, Tompa P. Intrinsic structural disorder confers cellular viability on oncogenic fusion proteins. *PLoS Comput Biol*. 2009;5(10):e1000552.
  31. Markiv A, Rambaruth ND, Dwek MV. Beyond the genome and proteome:

- targeting protein modifications in cancer. *Curr Opin Pharmacol.* 2012;12(4):408–413.
32. Peng Z, Mizianty MJ, Xue B, et al. More than just tails: intrinsic disorder in histone proteins. *Mol Biosyst.* 2012;8(7):1886–1901.
33. Weatheritt RJ, Gibson TJ. Linear motifs: lost in (pre)translation. *Trends Biochem Sci.* 2012;37(8):333–341.
34. Babu MM, van der Lee R, de Groot NS, et al. Intrinsically disordered proteins: regulation and disease. *Curr Opin Struc Biol.* 2011;21(3):432–440.
- **Overview about the importance of IDPs for signaling fidelity and drug targeting.**
35. Vendruscolo M. Enzymatic activity in disordered states of proteins. *Curr Opin Chem Biol.* 2010;14(5):671–675.
36. Schulenburg C, Hilvert D. Protein conformational disorder and enzyme catalysis. *Top Curr Chem.* 2013;337:41–67.
37. Smock RG, Blackburn ME, Gierasch LM. Conserved, disordered C terminus of DnaK enhances cellular survival upon stress and DnaK in vitro chaperone activity. *J Biol Chem.* 2011;286(36):31821–31829.
38. Tompa P, Csermely P. The role of structural disorder in the function of RNA and protein chaperones. *FASEB J.* 2004;18(11):1169–1175.
39. Kuznetsova IM, Turoverov KK, Uversky VN. What macromolecular crowding can do to a protein. *Int J Mol Sci.* 2014;15(12):23090–23140.
40. Greenwald J, Riek R. Biology of amyloid: structure, function, and regulation. *Structure.* 2010;18(10):1244–1260.
41. Nag S, Sarkar B, Bandyopadhyay A, et al. Nature of the amyloid-beta monomer and the monomer-oligomer equilibrium. *J Biol Chem.* 2011;286(16):13827–13833.
42. Chakrabortee S, Meersman F, Kaminski Schierle GS, et al. Catalytic and chaperone-like functions in an intrinsically disordered protein associated with desiccation tolerance. *Proc Natl Acad Sci USA.* 2010;107(37):16084–16089.
43. Uversky VN. Targeting intrinsically disordered proteins in neurodegenerative and protein dysfunction diseases: another illustration of the D(2) concept. *Expert Rev Proteomics.* 2010;7(4):543–564.
44. Midic U, Oldfield CJ, Dunker AK, et al. Protein disorder in the human diseasome: unfoldomics of human genetic diseases. *BMC Genomics.* 2009;10(Suppl 1):S12.
45. Uversky VN, Oldfield CJ, Midic U, et al. Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. *BMC Genomics.* 2009;10(Suppl 1):S7.
46. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature.* 2011;475(7356):324–332.
47. Lee L, Sakurai M, Matsuzaki S, et al. SUMO and Alzheimer's disease. *NeuroMolecular Med.* 2013;15(4):720–736.
48. Uversky VN. Wrecked regulation of intrinsically disordered proteins in diseases: pathogenicity of deregulated regulators. *Front Mol Biosci.* 2014;1:6.
49. Brown CJ, Lain S, Verma CS, et al. Awakening guardian angels: drugging the p53 pathway. *Nat Rev Cancer.* 2009;9(12):862–873.
50. Muller PA, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell.* 2014;25(3):304–317.
51. Collavin L, Lunardi A, Del Sal G. p53-family proteins and their regulators: hubs and spokes in tumor suppression. *Cell Death Differ.* 2010;17(6):901–911.
52. Nair SK, Burley SK. X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors. *Cell.* 2003;112(2):193–205.
53. Dang CV. c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol.* 1999;19(1):1–11.
54. Hammoudeh DI, Follis AV, Prochownik EV, et al. Multiple independent binding sites for small-molecule inhibitors on the oncoprotein c-Myc. *J Am Chem Soc.* 2009;131(21):7390–7401.
55. Cheng Y, LeGall T, Oldfield CJ, et al. Rational drug design via intrinsically disordered protein. *Trends Biotechnol.* 2006;24(10):435–442.
56. Abelev GI. Alpha-fetoprotein in ontogenesis and its association with malignant tumors. *Adv Cancer Res.* 1971;14:295–358.
57. Cooper GJ, Leighton B, Dimitriadis GD, et al. Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc Natl Acad Sci USA.* 1988;85(20):7763–7766.
58. Cheng Y, LeGall T, Oldfield CJ, et al. Abundance of intrinsic disorder in protein associated with cardiovascular disease. *Biochemistry.* 2006;45(35):10448–10460.
59. Derisbourg M, Leghay C, Chiappetta G, et al. Role of the Tau N-terminal region in microtubule stabilization revealed by new endogenous truncated forms. *Sci Rep.* 2015;5:9659.
60. Chen J, Kanai Y, Cowan NJ, et al. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. *Nature.* 1992;360(6405):674–677.
61. Kadavath H, Hofele RV, Biernat J, et al. Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. *Proc Natl Acad Sci USA.* 2015;112(24):7501–7506.
62. Kadavath H, Jaremko M, Jaremko L, et al. Folding of the Tau protein on microtubules. *Angew Chem.* 2015;54(35):10347–10351.
- **Study demonstrating the folding of specific regions in microtubule associated protein Tau upon binding to mega-Dalton sized microtubules.**
63. Sergeant N, Bretteville A, Hamdane M, et al. Biochemistry of Tau in Alzheimer's disease and related neurological disorders. *Expert Rev Proteomics.* 2008;5(2):207–224.
64. Mandelkow EM, Biernat J, Drewes G, et al. Tau domains, phosphorylation, and interactions with microtubules. *Neurobiol Aging.* 1995;16(3):355–362; discussion 62–3.
65. Cripps D, Thomas SN, Jeng Y, et al. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J Biol Chem.* 2006;281(16):10825–10838.
66. Cohen TJ, Guo JL, Hurtado DE, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat Commun.* 2011;2:252.
67. Buee L, Bussiere T, Buee-Scherrer V, et al. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev.* 2000;33(1):95–130.
68. Tepper K, Biernat J, Kumar S, et al. Oligomer formation of tau protein hyperphosphorylated in cells. *J Biol Chem.* 2014;289(49):34389–34407.

69. Lasagna-Reeves CA, Castillo-Carranza DL, Jackson GR, et al. Tau oligomers as potential targets for immunotherapy for Alzheimer's disease and tauopathies. *Curr Alzheimer Res.* 2011;8(6):659–665.
70. Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. *Annu Rev Neurosci.* 2001;24:1121–1159.
- **Comprehensive review on the role of protein Tau in neurodegenerative diseases.**
71. Irwin DJ, Lee VM, Trojanowski JQ. Parkinson's disease dementia: convergence of alpha-synuclein, tau and amyloid-beta pathologies. *Nat Rev Neurosci.* 2013;14(9):626–636.
72. Akoury E, Gajda M, Pickhardt M, et al. Inhibition of tau filament formation by conformational modulation. *J Am Chem Soc.* 2013;135(7):2853–2862.
73. Noble W, Planel E, Zehr C, et al. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci USA.* 2005;102(19):6990–6995.
74. Zhang B, Maiti A, Shively S, et al. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc Natl Acad Sci USA.* 2005;102(1):227–231.
75. Pul R, Dodel R, Stangel M. Antibody-based therapy in Alzheimer's disease. *Expert Opin Biol Ther.* 2011;11(3):343–357.
76. Davidson WS, Jonas A, Clayton DF, et al. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J Biol Chem.* 1998;273(16):9443–9449.
77. Auluck PK, Caraveo G, Lindquist S. alpha-Synuclein: membrane interactions and toxicity in Parkinson's disease. *Annu Rev Cell Dev Biol.* 2010;26:211–233.
78. Goedert M, Spillantini MG, Del Tredici K, et al. 100 years of Lewy pathology. *Nat Rev Neurol.* 2013;9(1):13–24.
79. Jimenez JL, Guijarro JI, Orlova E, et al. Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. *EMBO J.* 1999;18(4):815–821.
80. Wegmann S, Muller DJ, Mandelkow E. Investigating fibrillar aggregates of Tau protein by atomic force microscopy. *Methods Mol Biol.* 2012;849:169–183.
81. Gibbs EB, Showalter SA. Quantitative biophysical characterization of intrinsically disordered proteins. *Biochemistry.* 2015;54(6):1314–1326.
82. Galea CA, Nourse A, Wang Y, et al. Role of intrinsic flexibility in signal transduction mediated by the cell cycle regulator, p27 Kip1. *J Mol Biol.* 2008;376(3):827–838.
83. Ferreon AC, Moran CR, Gambin Y, et al. Single-molecule fluorescence studies of intrinsically disordered proteins. *Methods Enzymol.* 2010;472:179–204.
84. Ferreon AC, Moosa MM, Gambin Y, et al. Counteracting chemical chaperone effects on the single-molecule alpha-synuclein structural landscape. *Proc Natl Acad Sci USA.* 2012;109(44):17826–17831.
85. Krishnan R, Goodman JL, Mukhopadhyay S, et al. Conserved features of intermediates in amyloid assembly determine their benign or toxic states. *Proc Natl Acad Sci USA.* 2012;109(28):11172–11177.
86. Ferreon AC, Ferreon JC, Wright PE, et al. Modulation of allostery by protein intrinsic disorder. *Nature.* 2013;498(7454):390–394.
87. Lee T, Moran-Gutierrez CR, Deniz AA. Probing protein disorder and complexity at single-molecule resolution. *Semin Cell Dev Biol.* 2015;37:26–34.
88. Ferreon AC, Gambin Y, Lemke EA, et al. Interplay of alpha-synuclein binding and conformational switching probed by single-molecule fluorescence. *Proc Natl Acad Sci USA.* 2009;106(14):5645–5650.
89. Ferreon AC, Moran CR, Ferreon JC, et al. Alteration of the alpha-synuclein folding landscape by a mutation related to Parkinson's disease. *Angew Chem.* 2010;49(20):3469–3472.
90. Aznauryan M, Nettels D, Holla A, et al. Single-molecule spectroscopy of cold denaturation and the temperature-induced collapse of unfolded proteins. *J Am Chem Sci.* 2013;135(38):14040–14043.
91. Brucalé M, Schuler B, Samori B. Single-molecule studies of intrinsically disordered proteins. *Chem Rev.* 2014;114(6):3281–3317.
92. Soranno A, Koenig I, Borgia MB, et al. Single-molecule spectroscopy reveals polymer effects of disordered proteins in crowded environments. *Proc Natl Acad Sci USA.* 2014;111(13):4874–4879.
93. Wuttke R, Hofmann H, Nettels D, et al. Temperature-dependent solvation modulates the dimensions of disordered proteins. *Proc Natl Acad Sci USA.* 2014;111(14):5213–5218.
94. König I, Zarrine-Afsar A, Aznauryan M, et al. Single-molecule spectroscopy of protein conformational dynamics in live eukaryotic cells. *Nat Methods.* 2015;12(8):773–779.
95. Bernado P, Svergun DI. Structural analysis of intrinsically disordered proteins by small-angle X-ray scattering. *Mol Biosyst.* 2012;8(1):151–167.
96. Narayanan RL, Durr UH, Bibow S, et al. Automatic assignment of the intrinsically disordered protein Tau with 441-residues. *J Am Chem Sci.* 2010;132(34):11906–11907.
97. Felli IC, Pierattelli R. Recent progress in NMR spectroscopy: toward the study of intrinsically disordered proteins of increasing size and complexity. *IUBMB Life.* 2012;64(6):473–481.
- **Detailed description of advances in NMR spectroscopy for IDP characterization.**
98. Konrat R. NMR contributions to structural dynamics studies of intrinsically disordered proteins. *J Magn Reson.* 2014;241:74–85.
99. Yao X, Becker S, Zweckstetter M. A six-dimensional alpha proton detection-based APSY experiment for backbone assignment of intrinsically disordered proteins. *J Biomol NMR.* 2014;60(4):231–240.
100. Wishart DS, Sykes BD. The <sup>13</sup>C chemical-shift index: a simple method for the identification of protein secondary structure using <sup>13</sup>C chemical-shift data. *J Biomol NMR.* 1994;4(2):171–180.
101. Mittag T, Forman-Kay JD. Atomic-level characterization of disordered protein ensembles. *Curr Opin Struc Biol.* 2007;17(1):3–14.
- **Description of experimental and computational methods for IDP ensemble description.**
102. Jensen MR, Markwick PR, Meier S, et al. Quantitative determination of the conformational properties of partially folded and intrinsically disordered proteins using NMR dipolar couplings. *Structure.* 2009;17(9):1169–1185.
103. Kosol S, Contreras-Martos S, Cedeno C, et al. Structural characterization of

- intrinsically disordered proteins by NMR spectroscopy. *Molecules*. 2013;18(9):10802–10828.
104. Schwalbe M, Kadavath H, Biernat J, et al. Structural impact of Tau phosphorylation at threonine 231. *Structure*. 2015;23(8):1448–1458.
105. Salmon L, Nodet G, Ozenne V, et al. NMR characterization of long-range order in intrinsically disordered proteins. *J Am Chem Sci*. 2010;132(24):8407–8418.
106. Bertoncini CW, Jung YS, Fernandez CO, et al. Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein. *Proc Natl Acad Sci*. 2005;102(5):1430–1435.
- **Study revealing critical changes in conformational ensemble of alpha-synuclein, the protein which folds into pathogenic aggregates in Parkinson disease.**
107. Dedmon MM, Lindorff-Larsen K, Christodoulou J, et al. Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. *J Am Chem Sci*. 2005;127(2):476–477.
108. Schwalbe M, Ozenne V, Bibow S, et al. Predictive atomic resolution descriptions of intrinsically disordered hTau40 and alpha-synuclein in solution from NMR and small angle scattering. *Structure*. 2014;22(2):238–249.
109. Mukrasch MD, Bibow S, Korukottu J, et al. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biology*. 2009;7(2):e34.
- **Study reporting an ensemble description of the full-length Tau protein and its interactions with microtubules.**
110. Palmer AG, Massi F. Characterization of the dynamics of biomacromolecules using rotating-frame spin relaxation NMR spectroscopy. *Chem Rev*. 2006;106(5):1700–1719.
111. Korzhnev DM, Kay LE. Probing invisible, low-populated states of protein molecules by relaxation dispersion NMR spectroscopy: an application to protein folding. *Acc Chem Res*. 2008;41(3):442–451.
112. Allison JR, Varnai P, Dobson CM, et al. Determination of the free energy landscape of alpha-synuclein using spin label nuclear magnetic resonance measurements. *J Am Chem Sci*. 2009;131(51):18314–18326.
113. Xiang S, Gapsys V, Kim HY, et al. Phosphorylation drives a dynamic switch in serine/arginine-rich proteins. *Structure*. 2013;21(12):2162–2174.
- **Atomic level study of phosphorylation induced dynamic switch in an IDP.**
114. Lindorff-Larsen K, Trbovic N, Maragakis P, et al. Structure and dynamics of an unfolded protein examined by molecular dynamics simulation. *J Am Chem Sci*. 2012;134(8):3787–3791.
115. Piana S, Klepeis JL, Shaw DE. Assessing the accuracy of physical models used in protein-folding simulations: quantitative evidence from long molecular dynamics simulations. *Curr Opin Struc Biol*. 2014;24:98–105.
- **Assessment of molecular dynamic simulations for the study of IDPs.**
116. Patel S, Ramanujam V, Srivastava AK, et al. Conformational propensities and dynamics of a betagamma-crystallin, an intrinsically disordered protein. *Phys Chem Chem Phys*. 2014;16(25):12703–12718.
117. Rezaei-Ghaleh N, Klama F, Munari F, et al. Predicting the rotational tumbling of dynamic multidomain proteins and supramolecular complexes. *Angew Chem*. 2013;52(43):11410–11414.
118. Rezaei-Ghaleh N, Klama F, Munari F, et al. HYCUD: a computational tool for prediction of effective rotational correlation time in flexible proteins. *Bioinformatics*. 2015;31(8):1319–1321.
119. Metallo SJ. Intrinsically disordered proteins are potential drug targets. *Curr Opin Chem Biol*. 2010;14(4):481–488.
120. Follis AV, Hammoudeh DI, Wang H, et al. Structural rationale for the coupled binding and unfolding of the c-Myc oncoprotein by small molecules. *Chem Biol*. 2008;15(11):1149–1155.
121. Cuchillo R, Michel J. Mechanisms of small-molecule binding to intrinsically disordered proteins. *Biochem Soc Trans*. 2012;40(5):1004–1008.
122. Michel J, Cuchillo R. The impact of small molecule binding on the energy landscape of the intrinsically disordered protein C-myc. *PLoS One*. 2012;7(7):e41070.
123. Jin F, Yu C, Lai L, et al. Ligand clouds around protein clouds: a scenario of ligand binding with intrinsically disordered proteins. *PLoS Comput Biol*. 2013;9(10):e1003249.
124. Krishnan N, Koveal D, Miller DH, et al. Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. *Nat Chem Biol*. 2014;10(7):558–566.
125. Heller GT, Sormanni P, Vendruscolo M. Targeting disordered proteins with small molecules using entropy. *Trends Biochem Sci*. 2015;40(9):491–496.
126. Fonseca-Ornelas L, Eisbach SE, Paulat M, et al. Small molecule-mediated stabilization of vesicle-associated helical alpha-synuclein inhibits pathogenic misfolding and aggregation. *Nat Comm*. 2014;5:5857.
- **Study describing a novel strategy for inhibition of the pathogenic aggregation of vesical bound alpha-synuclein.**
127. Jung KY, Wang H, Teriete P, et al. Perturbation of the c-Myc-Max protein-protein interaction via synthetic alpha-helix mimetics. *J Med Chem*. 2015;58(7):3002–3024.
128. Schirmer RH, Adler H, Pickhardt M, et al. Lest we forget you—methylene blue. *Neurobiol Aging*. 2011;32(12):2325, e7–16.
129. Wischik CM, Staff RT, Wischik DJ, et al. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. *J Alzheimers Dis*. 2015;44(2):705–720.
130. Akoury E, Pickhardt M, Gajda M, et al. Mechanistic basis of phenothiazine-driven inhibition of Tau aggregation. *Angew Chem*. 2013;52(12):3511–3515.
- **NMR-based study elucidating the mechanism of small molecule-mediated inhibition of Tau aggregation.**
131. Toth G, Gardai SJ, Zago W, et al. Targeting the intrinsically disordered structural ensemble of alpha-synuclein by small molecules as a potential therapeutic strategy for Parkinson's disease. *PLoS One*. 2014;9(2):e87133.
- **First report on directly targeting IDP ensembles for drug discovery.**
132. Weinreb PH, Zhen W, Poon AW, et al. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry*. 1996;35(43):13709–13715.
133. Acharya S, Safaie BM, Wongkongkathep P, et al. Molecular basis for preventing alpha-synuclein aggregation by a molecular tweezer. *J Biol Chem*. 2014;289(15):10727–10737.
134. Prabhudesai S, Sinha S, Attar A, et al. A novel “molecular tweezer” inhibitor of



- alpha-synuclein neurotoxicity in vitro and in vivo. *Neurotherapeutics*. 2012;9(2):464–476.
135. Mangialasche F, Solomon A, Winblad B, et al. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol*. 2010;9(7):702–716.
136. Chene P. Inhibition of the p53-MDM2 interaction: targeting a protein-protein interface. *Mol Cancer Res*. 2004;2(1): 20–28.
137. Khoo KH, Verma CS, Lane DP. Drugging the p53 pathway: understanding the route to clinical efficacy. *Nat Rev Drug Discov*. 2014;13(3):217–236.
138. Yin X, Giap C, Lazo JS, et al. Low molecular weight inhibitors of Myc-Max interaction and function. *Oncogene*. 2003;22(40):6151–6159.
139. Aeluri M, Chmakuri S, Dasari B, et al. Small molecule modulators of protein-protein interactions: selected case studies. *Chem Rev*. 2014;114(9): 4640–4694.
140. Belmar J, Fesik SW. Small molecule Mcl-1 inhibitors for the treatment of cancer. *Pharmacol Ther*. 2015; 145:76–84.
141. Besbes S, Mirshahi M, Pocard M, et al. New dimension in therapeutic targeting of BCL-2 family proteins. *Oncotarget*. 2015;6(15):12862–12871.

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