REVIEW

New technologies to analyse protein function: an intrinsic disorder perspective [version 1; peer review: 2 approved]

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Abstract

Functions of intrinsically disordered proteins do not require structure. Such structure-independent functionality has melted away the classic rigid "lock and key" representation of structure-function relationships in proteins, opening a new page in protein science, where molten keys operate on melted locks and where conformational flexibility and intrinsic disorder, structural plasticity and extreme malleability, multifunctionality and binding promiscuity represent a new-fangled reality. Analysis and understanding of this new reality require novel tools, and some of the techniques elaborated for the examination of intrinsically disordered protein functions are outlined in this review.

Keywords

intrinsically disordered protein, intrinsically disordered protein region, protein function, structure-function continuum

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Introduction to the disorder-based functionality: melted locks and molten keys

For more than a hundred years, the dominant model describing the molecular mechanism of protein functionality was the classic structure–function paradigm. This paradigm considered protein function in light of the “lock and key” hypothesis, where a unique biological function of a protein was considered to be the consequence of the presence of a unique and highly organized structure in its active site and where, in order to exert a chemical effect on each other, both a substrate and an enzyme have specific geometric shapes that fit exactly into each other, like a key specifically and uniquely fits to a lock^{1,2}. In line with this hypothesis were numerous pieces of evidence generated by the crystal structures of proteins solved by x-ray diffraction, careful analysis of protein denaturation and unfolding, and many other observations, all indicating that specific functionality of a given protein is defined by a unique spatial positioning of its amino acid side chains and prosthetic groups, suggesting that such a specific spatial arrangement of functional groups in biologically active proteins is defined by their unique 3D structures predetermined by the unique amino acid sequences encoded in unique genes. These correlations were in line with the famous “one gene–one enzyme” hypothesis, where a gene encodes a single enzyme that affects a single step in a metabolic pathway³. It is recognized now that the aforementioned “one gene–one enzyme” hypothesis is an oversimplification, and numerous observations fail to fit into or be explained by this model⁴. Accumulated data challenged both the functional requirement of a unique structure in a biologically active protein and the absolute validity of the “one gene–one enzyme” conjecture, suggesting that the related paradigms should be changed^{5–8}. In line with these considerations, it is recognized now that the complexity of biological systems is determined by protein diversification and not by the existence of a large number of distinct genes each encoding a unique protein⁹. In fact, multiple means cause the dramatic and efficient increase in the size of a functional proteome in comparison with the size of a corresponding genome. These proteome-diversifying factors include the allelic variations (that is, single- or multiple-point mutations, insertions and deletions [indels], and single-nucleotide polymorphisms), different pre-translational mechanisms affecting genes (for example, production of numerous mRNA variants by the alternative splicing and mRNA editing), and changes induced in proteins by numerous post-translational modifications (PTMs)^{10–14}. The result of this multilevel diversification that combines allelic variations, pre-translational alterations, and PTMs is the generation of multiple proteoforms, which are distinct protein molecules with different structures and diverse functions, from a single gene¹⁵.

Furthermore, it is also recognized now that many protein functions do not require unique structure. These structure-less biologically active proteins carrying structure-independent functions are currently known as intrinsically disordered proteins (IDPs) or hybrid proteins containing ordered domains and IDP regions (IDPRs)^{5–8,16–22}. These proteins, which were originally considered unique exceptions to the “lock and key” rule, are extremely common in nature; all proteomes of living organisms

and viruses analysed so far possess noticeable levels of intrinsic disorder^{5,19,20,22–41}; and the penetrance of disorder increases with the increase in the organism complexity^{19,23–25,42}. As an example, the fraction of proteins predicted to have long IDPRs (that is, disordered regions exceeding 30 consecutive residues) increases from Bacteria and Archaea to Eukaryota^{23,24,26,28,43}. The increased amount of disorder in eukaryotes is attributed to the increased roles of their cellular signalling that often relies on IDPs/IDPRs^{5,6,8,18,44–47}. Also, just a small fraction of proteins with known crystal structures in the Protein Data Bank are entirely devoid of disorder^{48,49}. An important feature of IDPs/IDPRs is their exceptional spatiotemporal heterogeneity, where different regions of a given protein can be ordered (or disordered) to a different degree^{50,51}. Therefore, the overall structure of functional proteins represents a continuous spectrum of conformations with a different degree and depth of disorder⁵⁰, thereby generating a complex protein structural space that defines a structure-disorder continuum with no clear boundary between ordered and disordered proteins/regions⁵⁰. The presence of the aforementioned different levels and depths of intrinsic disorder delineates the mosaic structure of proteins, which typically contain foldons (that is, independently foldable regions), inducible foldons (disordered regions that can fold at interaction with a binding partner), morphing inducible foldons (disordered regions that can fold differently at interaction with a different binding partner), semi-foldons (IDPRs that are always in the semi-folded state), non-foldons (IDPRs with entropic chain activities), and unfoldons (or conditionally disordered protein regions, which, in order to become functional or to make a protein active, have to undergo order-to-disorder transition)⁵⁰. Obviously, the presence of intrinsic disorder and conformational flexibility in proteins contributes to their structural and functional heterogeneity, representing additional means for generating proteoforms⁵². In fact, since any protein exists as a dynamic conformational ensemble, members of which have different structures (their structural differences could be rather subtle, as in the case of ordered proteins, or rather substantial, as in the case of IDPs/IDPRs) and potentially different functions, it can be considered a basic (or intrinsic or conformational) proteoform. Such a conformational proteoform is different from the inducible proteoform that originates from the various alterations (PTMs, mutations, or consequences of alternative splicing) of the canonical protein sequence and that represents a mixture of these various forms. Obviously, since it also represents a structural ensemble, any member of the inducible or modified proteoform (that is, any mutated, modified, or alternatively spliced form) is itself a conformational proteoform⁵². Finally, since protein function, interaction with specific partners, or placement inside the natural cellular environment can also affect the structural ensemble of both basic and induced proteoforms, functionality per se can be considered a factor generating new functioning proteoforms. As a result, instead of being depicted as an oversimplified “one gene–one protein” view, the actual gene–protein relationship is much more complex, being described by the “one gene–many proteins–many functions” model^{52,53}. Therefore, a correlation between protein structure and function represents a “protein structure–function continuum”, where at any given moment, any given protein exists as a

dynamic conformational ensemble containing multiple proteoforms (conformational/basic, inducible/modified, and functioning) characterized by diverse structural features and various functions⁵².

Concluding this section, we need to emphasize that the presence of intrinsic disorder and conformational flexibility in proteins changed the rigid “lock and key” model proposed for the description of the general molecular mechanisms of protein function. Although “lock and key” (or its modification in a form of induced fit) can be used for the description of catalytic activities of some enzymes, many other protein functions (for example, recognition, regulation, signalling, and promiscuous binding) do not fit into this rigid view since, owing to the presence of disorder and flexibility, the locks are melted and the keys are molten. This also suggests that some novel approaches are needed to analyse intrinsic disorder-based functionality. The goal of this article is to shed some light on this problem by presenting the most recent advances in the analysis of protein disorder-based functionality.

Looking at the disorder-based functionality of proteins

Laboratory techniques for the analysis of protein–protein interactions

Traditional analysis of protein functionality was mostly centred on the development of means for accurate characterization of enzymatic activity or ligand binding (or both) of a protein *in vitro* and *in vivo* and development of related molecular mechanisms. Although enzymatic catalysis is not among the disorder-based protein functions, some of the techniques elaborated for the analysis of the interactivity of ordered proteins can be successfully used for the functional characterization of IDPs. Biophysical techniques that are typically used to study protein–partner interactions are designed either to investigate thermodynamics or kinetics (or both) of the binding or to characterize the structural changes associated with the interactions. Many of these techniques are suitable for the analysis of both order-based and disorder-based protein interactions although the IDP-centred interactions involve a variety of binding modes, ranging from the folding upon binding mechanism to the formation of dynamic fuzzy complexes. Thermodynamic-focused techniques for the analysis of protein–partner interactions include isothermal titration calorimetry^{54,55} and surface plasmon resonance (SPR)⁵⁶, whereas dissociation constants can be measured by dynamic light scattering⁵⁷ and analytical ultracentrifugation⁵⁸. All of these techniques can determine dissociation constants. In addition, SPR can determine k_{on} and k_{off} of binding events⁵⁶. Although, traditionally, the major technique for the analysis of binding-induced structural changes in proteins was x-ray crystallography, this tool provides a static 3D picture of a protein complex and therefore has rather limited application to IDPs/IDPRs (with the obvious exception of the cases when disordered protein or region folds at interaction with the specific partner). Among other experimental techniques for the analysis of binding-induced structural changes are small-angle x-ray scattering (SAXS)^{59,60}, single-molecule Förster resonance energy transfer (smFRET) (that analyses protein conformations without ensemble averaging and kinetics without

interference from asynchronous processes)^{61–65}, electron paramagnetic resonance (EPR)^{64,66,67}, and hydrogen/deuterium exchange (HDX) mass spectrometry^{68–71}. Although IDPs/IDPRs are commonly involved in transient protein–protein interactions (that is, interactions characterized by the K_D values in the micromolar to millimolar range), which are crucial for cell signalling, characterization of such interactions at the atomic-resolution level is rather challenging by the majority of conventional techniques. However, such interactions can be analysed by using solution nuclear magnetic resonance (NMR) spectroscopy^{72–76}, including diamagnetic and paramagnetic (for example, paramagnetic relaxation enhancement) techniques⁷⁷. Peculiarities of the application of NMR for the analysis of IDPs/IDPRs and disorder-based protein complexes are detailed in several recent reviews^{72,75}. Importantly, smFRET^{78,79} and NMR^{80–82} can be successfully used for the in-cell analysis of IDPs and their interactions. It was also pointed out that the most appropriate and eloquent description of the structure and dynamics of IDPs and IDP-based complexes could be achieved via the combined use of several aforementioned techniques, such as NMR, smFRET, and SAXS enhanced by the molecular dynamic simulations, since complementary experimental data from these techniques ensure important and meaningful constraints for computational simulations^{83,84}. In line with these developments, several groups are developing new approaches for the computational descriptions of disordered ensembles^{85–99}. Furthermore, an openly accessible database of structural ensembles of intrinsically disordered and unfolded proteins, pE-DB (<http://pedb.vib.be>), was created to promote the elaboration of novel modelling approaches and to allow a better understanding of disorder-based functionality^{100,101}.

Illustrating the remarkable power of NMR spectroscopy when applied to the functional and structural analysis of disorder-based interactions, a recent study provided a structural characterization of an intriguing complex formed between two IDPs: human histone H1 and its nuclear chaperone prothymosin- α ¹⁰². Although these proteins formed a highly specific complex with picomolar affinity, they completely retained their highly disordered nature, long-range flexibility, and overall highly dynamic character¹⁰². This complex is an extreme case of an IDP-driven polyelectrostatic binding mechanism proposed as a result of the NMR-based analysis of a complex between the polyvalent intrinsically disordered cyclin-dependent kinase inhibitor Sic1 and its ordered partner, SCF ubiquitin ligase subunit Cdc4¹⁰³. This Sic1–Cdc4 complex is held together by cumulative electrostatic interactions between the numerous phosphorylated sites of Sic1 and a single binding site of Cdc4; the binding strength is dependent on the phosphorylation degree of Sic1, and Sic1 remains largely disordered in its Cdc4-bound state¹⁰³.

Multivalent interactions between IDPs that are not accompanied by noticeable structural changes are directly linked to the biogenesis of the proteinaceous membrane-less organelles (PMLOs), which are abundant in cytoplasm, nucleus, and mitochondria of various cells and which play a number of important roles in the organization of various intracellular

processes^{104,105}. PMLOs are related to various biological processes compartmentalized in diverse regions of the cell¹⁰⁶, are able to facilitate and respond to various biological functions and stimuli¹⁰⁷, and therefore are considered important players in cellular life. PMLOs are highly dynamic but stable, protein-only or protein–nucleic acid assemblages characterized by cell size-dependent dimensions¹⁰⁸, whose structural integrity and biogenesis are exclusively determined by protein–protein, protein–RNA, or protein–DNA interactions or a combination of these^{109,110}. These liquid droplets are formed via the intracellular liquid–liquid phase transitions (LLPTs) or the intracellular liquid–liquid demixing phase separation^{108,111} initiated by the colocalization of molecules at high concentrations within a small cellular micro-domain^{112,113}. Biogenesis of PMLOs is a highly controllable and reversible process, and formation of PMLOs is triggered by changes in the concentrations of proteins undergoing LLPT, changes in the concentrations of specific small molecules or salts, changes in osmolarity, and changes in the pH or temperature (or both) of the solution or by various PTMs and alternative splicing of the phase-forming proteins, by the binding of these proteins to some definite partners, or by changes in other environmental conditions that affect the protein–protein or protein–nucleic acid interactions^{108,111,114–116}. PMLOs are very large (detectable by light microscope), liquid-like assemblages which are not covered by the membranes and whose components are involved in direct contact and exchange with the PMLO environment^{112,113}. As a result, PMLOs are characterized by liquid-like behaviour, being capable of wetting, dripping, and forming spherical structures upon fusion^{108,117–119}. Since proteins driving LLPTs are intrinsically disordered or contain IDPRs¹²⁰, PMLOs represent an intricate form of the disorder-based protein complexes^{104,105,121}, which are commonly formed without noticeable structural changes in the proteins undergoing LLPTs¹²². This conclusion is supported by the NMR analysis of several PMLOs or liquid droplets such as in the case of the Alzheimer-related protein tau^{123,124}, elastin-like polypeptides (ELPs)¹²⁵, the low-complexity domain of the RNA-binding protein fused in sarcoma (FUS)¹²⁶, heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2)¹²⁷, and the intrinsically disordered N-terminal 236 residues of the germ-granule protein Ddx4¹²⁸. Techniques that can be used for the analysis of the dynamics, structure, morphology, and rheology of phase-separated droplets and PMLOs and their components *in vitro* and in live cells were systematically analysed in a recent review¹²⁹. Special emphasis was put on the suitability of single-molecule fluorescence methods for the characterization of functional dynamics of PMLOs¹³⁰, on the use of fluorescence recovery after photobleaching (FRAP) as a technique of first choice for assessing fluidity of PMLOs and phase-separated droplets and to estimate protein diffusion coefficients¹³¹, and dual-colour fluorescence cross-correlation spectroscopy (FCCS) for the analysis of concentrations, diffusion characteristics and interactions of two fluorescent species in solution¹³².

The liquid-like nature of PMLOs and phase-separated droplets can affect and modulate functions of their constituents, which are accumulated within droplets at high concentrations but remain dynamic. In line with this hypothesis, the low-density

structure of PMLOs in the *Xenopus* oocyte nucleus was shown to determine the access to the macromolecules within these PMLOs from the nucleoplasm¹³³. PMLOs can also act as liquid-phase micro-reactors, where the cytoplasmic reactions are accelerated because of the increased concentrations of related RNA and protein components^{108,134,135}. PMLOs can also serve as a means for recruitment and concentration of specific proteins, as seen in Negri bodies (NBs), which are cytoplasmic liquid organelles in which viral RNAs (mRNAs as well as genomic and antigenomic RNAs) are synthesized¹³⁶. Neuronal ribonucleoprotein (RNP) particles, or granules that concentrate specific sets of mRNAs and regulatory proteins, serve as dynamic sensors of localized signals and play a dual role in the translation of associated mRNAs, preventing mRNA translation at rest and ensuring local protein synthesis upon activation¹³⁷.

LLPTs and PMLOs are illustrative examples of the disorder-based emergent behaviour of proteins^{50,138–140}. Another example of the emergent behaviour is given by the spatiotemporal oscillations of the Min protein system (MinD, MinC and MinE) that moves from pole to pole of the rod-shaped *Escherichia coli* cells with the intrinsic wavelength comparable to the size of the *E. coli* cell¹⁴¹. Oscillating movements of this system are required for the spatial regulation of the positioning of the cytokinetic Z ring that determines the division plane^{142–144}. Such oscillations can be visualized if the components of this system are fluorescently labelled^{145–147}. Furthermore, on the supported lipid bilayers *in vitro*, these Min proteins self-organize to form traveling protein surface waves emerging from the repetitive binding-detaching cycles of proteins to the membrane^{143,144,148,149}. Also, depending on the peculiarities of their environment, MinD and MinE were shown to self-organize into a wide variety of patterns¹⁵⁰.

Bioimaging is a commonly used technique for the quantification of intracellular protein–protein interactions (PPIs). Here, the presence of molecular interactions is judged by the analysis of spatial colocalization between the different populations of differently labelled molecules in the field of view (FOV) of dual- or multiple-channel fluorescence microscope¹⁵¹. Colocalization is evaluated by pixel-based methods or object-based methods¹⁵¹. In the first case, the image generated by the fluorescence microscope is analysed to measure global correlation coefficients between pixel intensities in different colour channels that allow finding and quantification of overlapping pixel intensities in different channels¹⁵². In the second case, the objects (molecules) are first segmented and then represented as points through coordinates of their mass centre in the delimited FOV and then their spatial distributions are analysed^{153,154}. A systematic study published in 2015 compared pixel-based and object-based methods for finding colocalization in synthetic and biological images and revealed that data generated by the object-based methods are more statistically robust than the results of pixel-based approaches¹⁵¹.

PPIs *in vitro* and *in vivo* are traditionally analysed by using the affinity purification-based pull-down assays¹⁵⁵ or co-immunoprecipitation (coIP) experiments¹⁵⁶ allowing the

direct detection of physical interactions. Here, either purified and tagged protein is used as a “bait” to bind any interacting proteins (pull-down assays) or antibody against a target protein is used to immunoprecipitate the complexes containing the target protein (coIP). Although coIP and pull-down assays are typically used as “yes-no” tools for showing the presence or absence of PPIs, it was recently shown that the dissociation constant (K_D) of complexes formed by two purified proteins can be measured by using the quantitative pull-down assay¹⁵⁷. However, these two techniques are typically limited to the high-affinity binding and therefore are not easily transferable to the analysis of disorder-based interactions, which are often weak. This caveat can be overcome by using chemical¹⁵⁸ or photo-affinity¹⁵⁹ cross-linking of samples before conducting pull-down and coIP assays. Chemical and photo-affinity cross-linking combined with mass spectrometry (XL-MS) is another technique for the analysis of weak and transient PPIs^{159–164}. The use of genetically encoded photo-crosslinkers using natural amino acid analogues that contain a photo-affinity group as the warhead and that can be site-specifically incorporated into a protein of interest to covalently trap non-covalent PPIs under living conditions represents a promising development in this area¹⁶⁵.

One of the commonly used approaches for investigating PPIs in living systems is a genetic approach: yeast-two-hybrid (YTH) screening^{166–168}. Here, interaction between two proteins, called bait and prey, activates reporter genes that enable yeast growth on specific media or a colour reaction¹⁶⁸. In 2015, high-affinity binders to transiently structured IDP, the prokaryotic ubiquitin-like protein Pup, and its unstructured segments were identified and characterized at atomic resolution by using the YTH-selected peptide aptamers and in-cell NMR¹⁶⁹. Similarly, a combination of YTH screenings with NMR spectroscopy, cross-linking experiments, and competition-binding assays was recently used to characterize the interactivity of a long IDPR linking the KIX domain (kinase-inducible domain [KID] interacting domain) and bromodomain of CBP (cAMP response element-binding [CREB]-binding protein) termed ID3 and to show that ID3 binds to the intrinsically disordered RNA-binding Zinc-finger protein 106 (ZFP106), and both interactors maintained disorder in their bound states¹⁷⁰. Recently, YTH assay was used to compare mutational robustness of the intrinsically disordered viral protein VPg and of its interactor eIF4E using libraries of mutant forms of both VPg and eIF4E¹⁷¹. This study revealed that VPg was significantly more robust against mutations than eIF4E¹⁷¹.

Another tool for the analysis of weak PPIs is the bimolecular fluorescence complementation (BiFC) assay, which uses the ability of two non-fluorescent fragments of a fluorescent protein to associate and form a fluorescent complex, and association is facilitated when they are fused to two interacting proteins^{172,173}. BiFC was successfully used for the *in planta* analysis of homo- and hetero-dimerization of the intrinsically disordered dehydrins from *Arabidopsis thaliana*, AtCOR47, AtERD10 and AtRAB18¹⁷⁴, and for the analysis of interactivity of another *Arabidopsis* protein, histone deacetylase complex 1 (HDC1) protein¹⁷⁵.

Finally, among other experimental tools used for the analysis of PPIs are various proximity-dependent labelling (PDL) approaches, where the target protein has to be fused with an enzyme capable of catalytic attachment of a reactive molecule to the interacting partners in a distance-dependent manner (typically a few tens to hundreds of nanometers)^{176–178}. One of these PDL systems is a proximity-dependent biotin identification (BioID) approach that uses biotin ligase BirA as an enzyme catalysing the biotinylation of target protein in the presence of biotin and that uses subsequent streptavidin-mediated pull-down and mass spectrometry analysis for the identification of interacting proteins^{179,180}. Recently, it was shown that biotinylation-based proximity labelling is biased by structural features of target proteins, causing enrichment of cellular biotinylation events within the IDPRs of protein targets¹⁸¹. In addition to biotin ligase, proximity labelling can be conducted by some peroxidase enzymes, which, in the presence hydrogen peroxide, can generate short-lived free radicals (for example, from phenolic compounds) that represent the enzyme-generated reagents that can covalently label neighbouring proteins^{178,182}.

Computational approaches for the analysis of disorder-based functionality

Among the important features of IDPs/IDPRs associated with their functionality are the ability to undergo at least partial folding at interaction with specific partners^{5,8,18,44–47,183–189} and the capability to bind to multiple partners and gain very different structures in the bound state^{190–196}, which increases complexity of the disorder-based interactomes¹⁹⁷. Often, such foldable IDPRs are engaged in recognition function of IDPs and therefore are known as molecular recognition features^{188,198–201}. Since such molecular recognition features (MoRFs) (for example, sub-regions of IDPs/IDPRs capable of binding-induced folding) are characterized by specific features (they cannot fold by themselves but have the potential to do so when a specific partner is present), they can be rather accurately predicted from the protein amino acid sequence²⁰². There are numerous computational tools for finding disorder-based interactions sites in proteins, which are grouped into three major classes: tools looking for MoRFs (alpha-MoRFpred^{188,200}, MoRFpred²⁰³, MFSPSSMpred²⁰⁴, MoRFChiBi^{205,206}, fMoRFpred²⁰⁷, retro-MoRF²⁰⁸, and DISOPRED3²⁰⁹); algorithms such as PepBindPred²¹⁰, ANCHOR^{211,212} and disorDPbind²¹³ to find generic disordered protein-binding regions; and a method for finding short linear sequence motifs (SLiMs), SLiMpred²¹⁴. Although all of these tools analyse the capability of a target protein to be engaged in PPIs, disorDPbind also predicts the protein region capable of binding to DNA and RNA²¹³. There is also a tool for finding disordered flexible linker regions that serve as linkers/spacers in multi-domain proteins or between structured constituents in protein domains: the DFLpred method²¹⁵. Peculiarities, advantages and disadvantages of all of these techniques, together with the 32 tools for the prediction of intrinsic disorder predisposition of a query protein, were carefully analysed and compared in a recent comprehensive review²⁰². Recently, Zarin *et al.* did a comprehensive evolutionary computational analysis to search for molecular features that are preserved in the amino acid sequences of orthologous IDPRs²¹⁶. This analysis revealed that orthologous IDPRs frequently

contain multiple “evolutionary signatures” (that is, molecular features, which are preserved within these IDPRs and are associated with multiple functional annotations and phenotypes). Based on these observations, it was suggested that such evolutionary signatures could be used for the prediction of functionality of IDPRs from their amino acid sequences²¹⁶.

Another important feature of disorder-based functions is their regulation by numerous PTMs^{5,6,44,45,217,218}. Therefore, prediction of localization of PTM sites within the amino acid sequences of IDPs and IDPRs represents an important direction in computational analysis of disorder-based functionality. In fact, systematic bioinformatic analyses of the peculiarities of the IDP/IDPR-located display sites targeted for PTMs and their adjacent regions demonstrated that their sequence attributes (such as amino acid compositions and sequence complexity, hydrophobicity, and charge) are rather similar to those of IDPRs. These observations define the potential predictability of such disorder-centred PTM sites and were used for the development of disorder-focused predictors of protein phosphorylation²¹⁷, methylation²¹⁹, ubiquitination²²⁰, and S-palmitoylation²²¹, a unified sequence-based predictor of 23 types of PTM sites, which can be used for finding protein regions that undergo multiple homologous or heterologous PTM events and for finding shared PTM sites (that is, sites modified by more than one type of PTM)²¹⁸.

Disorder status and potential disorder-related information for a query protein can be retrieved from the D²P² database (<http://d2p2.pro/>)²²², which is a resource of pre-computed disorder predictions for a large library of proteins from completely sequenced genomes²²². In a visually attractive form, D²P² generates a functional disorder profile of a query protein that includes outputs of nine per-residue disorder predictors, represents positions of functional domains, shows a gradient bar reflecting the consensus of nine disorder predictors, where the increase in strength of correlation is shown by colour change from white to dark green, and also indicates location of the predicted disorder-based binding sites (MoRFs) and positions of various PTMs²²².

Finally, localization of various functional short linear motifs, SLiMs, in a query protein can be assessed by the eukaryotic linear motif (ELM) resource (<http://elm.eu.org/>), which is a collection of manually annotated SLiM instances curated from experimental literature^{223,224}. SLiMs are composed of short stretches of adjacent amino acids and can be found in IDPRs of many proteins. They are short, compact, degenerate peptide segments that act as protein interaction sites and are essential for almost all cellular processes²²³. An ELM resource can also be used for finding potential SLiMs in a query protein. It filters out globular domains and retains predicted SLiMs associated with various functions^{223,224}. There are six types of annotations for the SLiMs that are described by the ELM server^{223,224}: motifs for targeting to subcellular compartments (ELM_TRG), degran motifs that play a role in polyubiquitylation and targeting of proteins to proteasomal degradation (ELM_DEG), motifs that act as proteolytic cleavage sites (ELM_CLV), ligand binding

motifs (ELM_LIG), docking motifs (ELM_DOC), and sites for PTMs (ELM_MOD)^{223,224}.

One more important recent direction in the elaboration of computational tools for functional analysis of IDPs and IDPRs is related to the development of methods for prediction of liquid-liquid phase separation (LLPS) and finding phase-separating proteins (PSPs). In fact, although the analysis of LLPTs and PMLOs is a rapidly developing field that clearly attracts significant attention of multiple researchers, general knowledge of the prevalence and distribution of PSPs is still rather rudimentary. Therefore, tools for LLPS and PSP predictions are in high demand. Recently, information on the first-generation PSP predictors and their basic principles was summarized by Vernon *et al.*²²⁵. Among these first-generation PSP predictors are the following:

- Prion-like amino acid composition (PLAAC) tool for finding PSPs²²⁶ on the basis of identifying prion-like domains²²⁷;
- A tool for finding PSPs on the basis of the similarity of sequence composition and residue spacing (statistical map of FG and RG) to fingerprints of PMLO-forming features of a disordered nuage protein Ddx4¹²²;
- PScore that evaluates the expected number of long-range π - π interactions involving non-aromatic groups in a query protein²²⁸;
- LARKS tool for finding, in query proteins, low-complexity aromatic-rich kinked segments that are potentially related to the formation of subcellular membrane-less assemblies²²⁹;
- R+Y model for the evaluation of the content in a query protein of arginine and tyrosine residues that can be engaged in cation- π interactions serving as drivers of phase separation²³⁰;
- the *cat*GRANULE algorithm that predicts PCPs by evaluating intrinsic disorder and nucleic acid binding propensities; sequence length; and arginine, glycine and phenylalanine content (R, G, F), which are known to be enriched in some PCPs²³¹;
- PSPer uses the hidden Markov model for prediction of PSPs and considers the presence in a query protein of prion-like domains, IDPRs, arginine-rich domains, RNA recognition motifs, and other features²³²;
- PSPredictor, which is a machine learning tool for sequence-based prediction of PSPs²³³.

Another illustration of the interest of researchers in LLPS and PMLOs is the recent development of an LLPDB database (<http://bio-comp.org.cn/llpsdb>) that provides comprehensive information on proteins undergoing LLPS *in vitro* and contains 1182 entries describing 273 independent proteins and 2394 specific conditions²³⁴.

Concluding remarks

Although IDPs/IDPRs were largely ignored for most of the existence of protein science, it is now clear that IDPs and disorder-based functions represent a new reality. Originally, the field of un-structural biology stood up as an attempt to explain many cases of rare exceptions (that is, proteins that fall outside of the classic structure–function paradigm with its “rigid” view of protein functionality as “lock and key” or “induced fit” models). However, in light of the broad acceptance of the new un-structural biology paradigm, one should keep in mind that it would be a clear mistake to continue contradicting and opposing ordered proteins and IDPs, as they work together in a living cell, indicating that understanding and explanation of the protein dynamics and functionality require a tandem action of the disciplines of structural and un-structural biology²³⁵. In fact, since different disorder-centred functions complement (mostly catalytic) activities of ordered proteins, structure and disorder represent a unity of opposites or *coincidentia oppositorum*. On the other hand, an actual line between order and disorder is elusive and structural and un-structural biology should not be opposed but united since they clearly complement one other²³⁵. Therefore, a complete understanding of the biological functionality at the proteome level requires careful consideration of both order- and disorder-based protein functions and only such a united approach can ensure the previously unattainable

comprehension of biological complexity. On the other hand, structural and functional characterization of ordered and disordered proteins requires very different methodological approaches, and an analysis of hybrid proteins remains a challenging task. In fact, as was pointed out, the current literature is focused mostly on fully ordered or fully disordered proteins, generating an immense “grey” area, where order and disorder are mixed and resulting in an incomplete understanding of the diverse mechanisms and functions used by hybrid proteins²³⁵.

Abbreviations

BiFC, bimolecular fluorescence complementation; coIP, co-immunoprecipitation; ELM, eukaryotic linear motif; FOV, field of view; ID3, IDPR linking the KIX domain and bromodomain of CBP; IDP, intrinsically disordered protein; IDPR, intrinsically disordered protein region; LLPS, liquid–liquid phase separation; LLPT, liquid–liquid phase transition; MoRF, molecular recognition feature; NMR, nuclear magnetic resonance; PDL, proximity-dependent labelling; PMLO, proteinaceous membrane-less organelle; PPI, protein–protein interaction; PSP, phase-separating protein; PTM, post-translational modification; SAXS, small-angle x-ray scattering; SLiM, short linear sequence motif; smFRET single-molecule Förster resonance energy transfer; SPR, surface plasmon resonance; YTH, yeast-two-hybrid

References

- Fischer E: **Influence of the configuration on the effect of the enzymes.** *Ber Dtsch Chem Ges.* 1894; **27**(3): 2985–2993.
[Publisher Full Text](#)
- Lemieux RU, Spohr U: **How Emil Fischer was led to the lock and key concept for enzyme specificity.** *Adv Carbohydr Chem Biochem.* 1994; **50**: 1–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Beadle GW, Tatum EL: **Genetic Control of Biochemical Reactions in Neurospora.** *Proc Natl Acad Sci U S A.* 1941; **27**(11): 499–506.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bussard AE: **A scientific revolution? The prion anomaly may challenge the central dogma of molecular biology.** *EMBO Rep.* 2005; **6**(8): 691–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dunker AK, Lawson JD, Brown CJ, *et al.*: **Intrinsically disordered protein.** *J Mol Graph Model.* 2001; **19**(1): 26–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tompa P: **Intrinsically unstructured proteins.** *Trends Biochem Sci.* 2002; **27**(10): 527–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Uversky VN, Gillespie JR, Fink AL: **Why are “natively unfolded” proteins unstructured under physiologic conditions?** *Proteins.* 2000; **41**(3): 415–27.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wright PE, Dyson HJ: **Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm.** *J Mol Biol.* 1999; **293**(2): 321–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schlüter H, Apweiler R, Holzhütter HG, *et al.*: **Finding one's way in proteomics: a protein species nomenclature.** *Chem Cent J.* 2009; **3**: 11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uhlén M, Björling E, Agaton C, *et al.*: **A human protein atlas for normal and cancer tissues based on antibody proteomics.** *Mol Cell Proteomics.* 2005; **4**(12): 1920–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Farrah T, Deutsch EW, Omenn GS, *et al.*: **State of the human proteome in 2013 as viewed through PeptideAtlas: comparing the kidney, urine, and plasma proteomes for the biology- and disease-driven Human Proteome Project.** *J Proteome Res.* 2014; **13**(1): 60–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Farrah T, Deutsch EW, Hoopmann MR, *et al.*: **The state of the human proteome in 2012 as viewed through PeptideAtlas.** *J Proteome Res.* 2013; **12**(1): 162–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Reddy PJ, Ray S, Srivastava S: **The quest of the human proteome and the missing proteins: digging deeper.** *OMICS.* 2015; **19**(5): 276–82.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kim MS, Pinto SM, Getnet D, *et al.*: **A draft map of the human proteome.** *Nature.* 2014; **509**(7502): 575–81.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Smith LM, Kelleher NL: **Proteomeform: a single term describing protein complexity.** *Nat Methods.* 2013; **10**(3): 186–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uversky VN: **Natively unfolded proteins: a point where biology waits for physics.** *Protein Sci.* 2002; **11**(4): 739–56.
[PubMed Abstract](#) | [Free Full Text](#)
- Uversky VN: **What does it mean to be natively unfolded?** *Eur J Biochem.* 2002; **269**(1): 2–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dyson HJ, Wright PE: **Intrinsically unstructured proteins and their functions.** *Nat Rev Mol Cell Biol.* 2005; **6**(3): 197–208.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Uversky VN: **The mysterious unfoldome: structureless, underappreciated, yet vital part of any given proteome.** *J Biomed Biotechnol.* 2010; **2010**: 568068.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uversky VN, Dunker AK: **Understanding protein non-folding.** *Biochim Biophys Acta.* 2010; **1804**(6): 1231–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dunker AK, Oldfield CJ, Meng J, *et al.*: **The unfoldomics decade: an update on intrinsically disordered proteins.** *BMC Genomics.* 2008; **9** Suppl 2: S1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dunker AK, Silman I, Uversky VN, *et al.*: **Function and structure of inherently disordered proteins.** *Curr Opin Struct Biol.* 2008; **18**(6): 756–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dunker AK, Obradovic Z, Romero P, *et al.*: **Intrinsic protein disorder in complete**

- genomes. *Genome Inform Ser Workshop Genome Inform.* 2000; **11**: 161–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. 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): 635–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Xue B, Dunker AK, Uversky VN: **Orderly order in protein intrinsic disorder distribution: disorder in 3500 proteomes from viruses and the three domains of life.** *J Biomol Struct Dyn.* 2012; **30**(2): 137–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Peng Z, Yan J, Fan X, *et al.*: **Exceptionally abundant exceptions: comprehensive characterization of intrinsic disorder in all domains of life.** *Cell Mol Life Sci.* 2015; **72**(1): 137–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
 27. Tokuriki N, Oldfield CJ, Uversky VN, *et al.*: **Do viral proteins possess unique biophysical features?** *Trends Biochem Sci.* 2009; **34**(2): 53–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Xue B, Williams RW, Oldfield CJ, *et al.*: **Archaic chaos: intrinsically disordered proteins in Archaea** *BMC Syst Biol.* 2010; **4** Suppl 1: S1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. Tompa P, Dosztanyi Z, Simon I: **Prevalent structural disorder in *E. coli* and *S. cerevisiae* proteomes.** *J Proteome Res.* 2006; **5**(8): 1996–2000.
[PubMed Abstract](#) | [Publisher Full Text](#)
 30. Krasowski MD, Reschly EJ, Ekins S: **Intrinsic disorder in nuclear hormone receptors.** *J Proteome Res.* 2008; **7**(10): 4359–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 31. Shimizu K, Toh H: **Interaction between Intrinsically disordered proteins frequently occurs in a human protein-protein interaction network.** *J Mol Biol.* 2009; **392**(5): 1253–65.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 32. Pentony MM, Jones DT: **Modularity of intrinsic disorder in the human proteome.** *Proteins.* 2010; **78**(1): 212–21.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Tompa P, Kalmar L: **Power law distribution defines structural disorder as a structural element directly linked with function.** *J Mol Biol.* 2010; **403**(3): 346–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
 34. Schad E, Tompa P, Hegyi H: **The relationship between proteome size, structural disorder and organism complexity.** *Genome Biol.* 2011; **12**(12): R120.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. Dyson HJ: **Expanding the proteome: disordered and alternatively folded proteins.** *Q Rev Biophys.* 2011; **44**(4): 467–518.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 36. Pancsa R, Tompa P: **Structural disorder in eukaryotes.** *PLoS One.* 2012; **7**(4): e34687.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 37. Midic U, Obradovic Z: **Intrinsic disorder in putative protein sequences.** *Proteome Sci.* 2012; **10**(Suppl 1): S19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 38. Hegyi H, Tompa P: **Increased structural disorder of proteins encoded on human sex chromosomes.** *Mol Biosyst.* 2012; **8**(1): 229–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
 39. Korneta I, Bujnicki JM: **Intrinsic disorder in the human spliceosomal proteome.** *PLoS Comput Biol.* 2012; **8**(8): e1002641.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 40. Kahali B, Ghosh TC: **Disorderiness in *Escherichia coli* proteome: perception of folding fidelity and protein-protein interactions.** *J Biomol Struct Dyn.* 2013; **31**(5): 472–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 41. Di Domenico T, Walsh I, Tosatto CE: **Analysis and consensus of currently available intrinsic protein disorder annotation sources in the MobiDB database.** *BMC Bioinformatics.* 2013; **14**(Suppl 7): S3.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 42. Oldfield CJ, Cheng Y, Cortese MS, *et al.*: **Comparing and combining predictors of mostly disordered proteins.** *Biochemistry.* 2005; **44**(6): 1989–2000.
[PubMed Abstract](#) | [Publisher Full Text](#)
 43. Na I, Redmon D, Kopa M, *et al.*: **Ordered disorder of the astrocytic dystrophin-associated protein complex in the norm and pathology.** *PLoS One.* 2013; **8**(8): e73476.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. Dunker AK, Brown CJ, Lawson JD, *et al.*: **Intrinsic disorder and protein function.** *Biochemistry.* 2002; **41**(21): 6573–82.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Dunker AK, Brown CJ, Obradovic Z: **Identification and functions of usefully disordered proteins.** *Adv Protein Chem.* 2002; **62**: 25–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
 46. Dunker AK, Obradovic Z: **The protein trinity—linking function and disorder.** *Nat Biotechnol.* 2001; **19**(9): 805–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Dyson HJ, Wright PE: **Coupling of folding and binding for unstructured proteins.** *Curr Opin Struct Biol.* 2002; **12**(1): 54–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
 48. Obradovic Z, Peng K, Vucetic S, *et al.*: **Predicting intrinsic disorder from amino acid sequence.** *Proteins.* 2003; **53**(Suppl 6): 566–72.
[PubMed Abstract](#) | [Publisher Full Text](#)
 49. DeForte S, Uversky VN: **Resolving the ambiguity: Making sense of intrinsic disorder when PDB structures disagree.** *Protein Sci.* 2016; **25**(3): 676–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 50. Uversky VN: **Unusual biophysics of intrinsically disordered proteins.** *Biochim Biophys Acta.* 2013; **1834**(5): 932–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
 51. Uversky VN: **A decade and a half of protein intrinsic disorder: biology still waits for physics.** *Protein Sci.* 2013; **22**(6): 693–724.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Uversky VN: **(Intrinsically disordered) splice variants in the proteome: Implications for novel drug discovery.** *Genes Genom.* 2016; **38**(7): 577–594.
[Publisher Full Text](#)
 53. Uversky VN: **p53 Proteoforms and Intrinsic Disorder: An Illustration of the Protein Structure-Function Continuum Concept.** *Int J Mol Sci.* 2016; **17**(11): pii: E1874.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. Velazquez-Campoy A, Leavitt SA, Freire E: **Characterization of protein-protein interactions by isothermal titration calorimetry.** *Methods Mol Biol.* 2015; **1278**: 183–204.
[PubMed Abstract](#) | [Publisher Full Text](#)
 55. Pierce MM, Raman CS, Nall BT: **Isothermal titration calorimetry of protein-protein interactions.** *Methods.* 1999; **19**(2): 213–21.
[PubMed Abstract](#) | [Publisher Full Text](#)
 56. Berggård T, Linse S, James P: **Methods for the detection and analysis of protein-protein interactions.** *Proteomics.* 2007; **7**(16): 2833–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
 57. Lorber B, Fischer F, Bailly M, *et al.*: **Protein analysis by dynamic light scattering: methods and techniques for students.** *Biochem Mol Biol Educ.* 2012; **40**(6): 372–82.
[PubMed Abstract](#) | [Publisher Full Text](#)
 58. Cole JL, Correia JJ, Stafford WF: **The use of analytical sedimentation velocity to extract thermodynamic linkage.** *Biophys Chem.* 2011; **159**(1): 120–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 59. Cordeiro TN, Herranz-Trillo F, Urbanek A, *et al.*: **Structural Characterization of Highly Flexible Proteins by Small-Angle Scattering.** *Adv Exp Med Biol.* 2017; **1009**: 107–29.
[PubMed Abstract](#) | [Publisher Full Text](#)
 60. Cordeiro TN, Herranz-Trillo F, Urbanek A, *et al.*: **Small-angle scattering studies of intrinsically disordered proteins and their complexes.** *Curr Opin Struct Biol.* 2017; **42**: 15–23.
[PubMed Abstract](#) | [Publisher Full Text](#)
 61. Ward RJ, Milligan G: **Structural and biophysical characterisation of G protein-coupled receptor ligand binding using resonance energy transfer and fluorescent labelling techniques.** *Biochim Biophys Acta.* 2014; **1838**(1 Pt A): 3–14.
[PubMed Abstract](#) | [Publisher Full Text](#)
 62. Moosa MM, Ferreon AC, Deniz AA: **Forced folding of a disordered protein accesses an alternative folding landscape.** *ChemPhysChem.* 2015; **16**(1): 90–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 63. Moparthi SB, Thieulin-Pardo G, de Torres J, *et al.*: **FRET analysis of CP12 structural interplay by GAPDH and PRK.** *Biochem Biophys Res Commun.* 2015; **458**(3): 488–493.
[PubMed Abstract](#) | [Publisher Full Text](#)
 64. Na JH, Lee WK, Kim Y, *et al.*: **Biophysical characterization of the structural change of Nopp140, an intrinsically disordered protein, in the interaction with CK2 α .** *Biochem Biophys Res Commun.* 2016; **477**(2): 181–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 65. Wu S, Wang D, Liu J, *et al.*: **The Dynamic Multisite Interactions between Two Intrinsically Disordered Proteins.** *Angew Chem Int Ed Engl.* 2017; **56**(26): 7515–7519.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 66. Papaioannou D, Geibel S, Kunze MB, *et al.*: **Structural and biophysical investigation of the interaction of a mutant Grb2 SH2 domain (W121G) with its cognate phosphopeptide.** *Protein Sci.* 2016; **25**(3): 627–37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 67. Bagnris C, Rogala KB, Baratchian M, *et al.*: **Probing the Solution Structure of IB Kinase (IKK) Subunit and Its Interaction with Kaposi Sarcoma-associated Herpes Virus Flice-interacting Protein and IKK Subunit by EPR Spectroscopy.** *J Biol Chem.* 2015; **290**(27): 16539–49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 68. Balasubramanian D, Komives EA: **Hydrogen-exchange mass spectrometry for the study of intrinsic disorder in proteins.** *Biochim Biophys Acta.* 2013; **1834**(6): 1202–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 69. Resetca D, Wilson DJ: **Characterizing rapid, activity-linked conformational transitions in proteins via sub-second hydrogen deuterium exchange mass spectrometry.** *FEBS J.* 2013; **280**(22): 5616–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
 70. Oganessian I, Lento C, Wilson DJ: **Contemporary hydrogen deuterium exchange mass spectrometry.** *Methods.* 2018; **144**: 27–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)

71. Sinz A: **Cross-Linking/Mass Spectrometry for Studying Protein Structures and Protein-Protein Interactions: Where Are We Now and Where Should We Go from Here?** *Angew Chem Int Ed Engl.* 2018; **57**(22): 6390–6396.
[PubMed Abstract](#) | [Publisher Full Text](#)
72. Teillum K, Kunze MB, Erlendsson S, *et al.*: **(S)Pinning down protein interactions by NMR.** *Protein Sci.* 2017; **26**(3): 436–451.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
73. Barandun J, Damberger FF, Delley CL, *et al.*: **Prokaryotic ubiquitin-like protein remains intrinsically disordered when covalently attached to proteasomal target proteins.** *BMC Struct Biol.* 2017; **17**(1): 1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
74. Bessa LM, Launay H, Dujardin M, *et al.*: **NMR reveals the intrinsically disordered domain 2 of NSSA protein as an allosteric regulator of the hepatitis C virus RNA polymerase NSSB.** *J Biol Chem.* 2017; **292**(44): 18024–18043.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
75. Prestel A, Bugge K, Staby L, *et al.*: **Characterization of Dynamic IDP Complexes by NMR Spectroscopy.** *Methods Enzymol.* 2018; **611**: 193–226.
[PubMed Abstract](#) | [Publisher Full Text](#)
76. Tolchard J, Walpole SJ, Miles AJ, *et al.*: **The intrinsically disordered Tarp protein from chlamydia binds actin with a partially preformed helix.** *Sci Rep.* 2018; **8**(1): 1960.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
77. Liu Z, Gong Z, Dong X, *et al.*: **Transient protein–protein interactions visualized by solution NMR.** *Biochim Biophys Acta.* 2016; **1864**(1): 115–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
78. Fakhree MAA, Noltén IS, Blum C, *et al.*: **Different Conformational Subensembles of the Intrinsically Disordered Protein α -Synuclein in Cells.** *J Phys Chem Lett.* 2018; **9**(6): 1249–1253.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
79. **F** Larsen EK, Weber DK, Wang S, *et al.*: **Intrinsically disordered HAX-1 regulates Ca^{2+} cycling by interacting with lipid membranes and the phospholamban cytoplasmic region.** *Biochim Biophys Acta Biomembr.* 2020; **1862**(1): 183034.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
80. Milles S, Salvi N, Blackledge M, *et al.*: **Characterization of intrinsically disordered proteins and their dynamic complexes: From *in vitro* to cell-like environments.** *Prog Nucl Magn Reson Spectrosc.* 2018; **109**: 79–100.
[PubMed Abstract](#) | [Publisher Full Text](#)
81. Wall KP, Hough LE: **In-Cell NMR within Budding Yeast Reveals Cytoplasmic Masking of Hydrophobic Residues of FG Repeats.** *Biophys J.* 2018; **115**(9): 1690–1695.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
82. Breindel L, Burz DS, Shekhtman A: **Interaction proteomics by using in-cell NMR spectroscopy.** *J Proteomics.* 2019; **191**: 202–211.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
83. **F** Gomes GN, Gradinaru CC: **Insights into the conformations and dynamics of intrinsically disordered proteins using single-molecule fluorescence.** *Biochim Biophys Acta Proteins Proteom.* 2017; **1865**(1 Pt B): 1696–1706.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
84. Liu Z, Gong Z, Cao Y, *et al.*: **Characterizing Protein Dynamics with Integrative Use of Bulk and Single-Molecule Techniques.** *Biochemistry.* 2018; **57**(3): 305–313.
[PubMed Abstract](#) | [Publisher Full Text](#)
85. **F** da Silva Neto AM, Silva SR, Vendruscolo M, *et al.*: **A superposition free method for protein conformational ensemble analyses and local clustering based on a differential geometry representation of backbone.** *Proteins.* 2019; **87**(4): 302–312.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
86. **F** Rangan R, Bonomi M, Heller GT, *et al.*: **Determination of Structural Ensembles of Proteins: Restraining vs Reweighting.** *J Chem Theory Comput.* 2018; **14**(12): 6632–6641.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
87. Heller GT, Bonomi M, Vendruscolo M: **Structural Ensemble Modulation upon Small-Molecule Binding to Disordered Proteins.** *J Mol Biol.* 2018; **430**(16): 2288–2292.
[PubMed Abstract](#) | [Publisher Full Text](#)
88. Bonomi M, Heller GT, Camilloni C, *et al.*: **Principles of protein structural ensemble determination.** *Curr Opin Struct Biol.* 2017; **42**: 106–16.
[PubMed Abstract](#) | [Publisher Full Text](#)
89. Bonomi M, Camilloni C, Vendruscolo M: **Metadynamic meta-inference: Enhanced sampling of the meta-inference ensemble using metadynamics.** *Sci Rep.* 2016; **6**: 31232.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
90. Fu B, Vendruscolo M: **Structure and Dynamics of Intrinsically Disordered Proteins.** *Adv Exp Med Biol.* 2015; **870**: 35–48.
[PubMed Abstract](#) | [Publisher Full Text](#)
91. Salvi N, Abyzov A, Blackledge M: **Multi-Timescale Dynamics in Intrinsically Disordered Proteins from NMR Relaxation and Molecular Simulation.** *J Phys Chem Lett.* 2016; **7**(13): 2483–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
92. Kragelj J, Blackledge M, Jensen MR: **Ensemble Calculation for Intrinsically Disordered Proteins Using NMR Parameters.** *Adv Exp Med Biol.* 2015; **870**: 123–47.
[PubMed Abstract](#) | [Publisher Full Text](#)
93. **F** Parigi G, Rezaei-Ghaleh N, Giachetti A, *et al.*: **Long-range correlated dynamics in intrinsically disordered proteins.** *J Am Chem Soc.* 2014; **136**(46): 16201–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
94. Kragelj J, Ozenne V, Blackledge M, *et al.*: **Conformational propensities of intrinsically disordered proteins from NMR chemical shifts.** *Chemphyschem.* 2013; **14**(13): 3034–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
95. **F** Jensen MR, Ruigrok RW, Blackledge M: **Describing intrinsically disordered proteins at atomic resolution by NMR.** *Curr Opin Struct Biol.* 2013; **23**(3): 426–35.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
96. Rezaei-Ghaleh N, Blackledge M, Zweckstetter M: **Intrinsically disordered proteins: from sequence and conformational properties toward drug discovery.** *ChemBiochem.* 2012; **13**(7): 930–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
97. Krzeminski M, Marsh JA, Neale C, *et al.*: **Characterization of disordered proteins with ENSEMBLE.** *Bioinformatics.* 2013; **29**(3): 398–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
98. Marsh JA, Teichmann SA, Forman-Kay JD: **Probing the diverse landscape of protein flexibility and binding.** *Curr Opin Struct Biol.* 2012; **22**(5): 643–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
99. Marsh JA, Forman-Kay JD: **Ensemble modeling of protein disordered states: experimental restraint contributions and validation.** *Proteins.* 2012; **80**(2): 556–72.
[PubMed Abstract](#) | [Publisher Full Text](#)
100. 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.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
101. Varadi M, Tompa P: **The Protein Ensemble Database.** *Adv Exp Med Biol.* 2015; **870**: 335–49.
[PubMed Abstract](#) | [Publisher Full Text](#)
102. **F** Borgia A, Borgia MB, Bugge K, *et al.*: **Extreme disorder in an ultrahigh-affinity protein complex.** *Nature.* 2018; **555**(7694): 61–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
103. **F** Borg M, Mittag T, Pawson T, *et al.*: **Polyelectrostatic interactions of disordered ligands suggest a physical basis for ultrasensitivity.** *Proc Natl Acad Sci U S A.* 2007; **104**(23): 9650–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
104. Uversky VN: **Intrinsically disordered proteins in overcrowded milieu: Membrane-less organelles, phase separation, and intrinsic disorder.** *Curr Opin Struct Biol.* 2017; **44**: 18–30.
[PubMed Abstract](#) | [Publisher Full Text](#)
105. Uversky VN: **Protein intrinsic disorder-based liquid-liquid phase transitions in biological systems: Complex coacervates and membrane-less organelles.** *Adv Colloid Interface Sci.* 2017; **239**: 97–114.
[PubMed Abstract](#) | [Publisher Full Text](#)
106. Forman-Kay JD, Kriwacki RW, Seydoux G: **Phase Separation in Biology and Disease.** *J Mol Biol.* 2018; **430**(23): 4603–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
107. Shin Y, Brangwynne CP: **Liquid phase condensation in cell physiology and disease.** *Science.* 2017; **357**(6357): pii: eaaf4382.
[PubMed Abstract](#) | [Publisher Full Text](#)
108. Feric M, Brangwynne CP: **A nuclear F-actin scaffold stabilizes ribonucleoprotein droplets against gravity in large cells.** *Nat Cell Biol.* 2013; **15**(10): 1253–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
109. Dunder M, Misteli T: **Biogenesis of nuclear bodies.** *Cold Spring Harb Perspect Biol.* 2010; **2**(12): a000711.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
110. Mao YS, Zhang B, Spector DL: **Biogenesis and function of nuclear bodies.** *Trends Genet.* 2011; **27**(8): 295–306.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
111. Uversky VN, Kuznetsova IM, Turoverov KK, *et al.*: **Intrinsically disordered proteins as crucial constituents of cellular aqueous two phase systems and coacervates.** *FEBS Lett.* 2015; **589**(1): 15–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
112. Phair RD, Misteli T: **High mobility of proteins in the mammalian cell nucleus.** *Nature.* 2000; **404**(6778): 604–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
113. Pederson T: **Protein mobility within the nucleus—what are the right moves?** *Cell.* 2001; **104**(5): 635–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
114. **F** Zhu L, Brangwynne CP: **Nuclear bodies: the emerging biophysics of nucleoplasmic phases.** *Curr Opin Cell Biol.* 2015; **34**: 23–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
115. Brangwynne CP, Tompa P, Pappu RV: **Polymer physics of intracellular phase transitions.** *Nature Phys.* 2015; **11**(11): 899–904.
[Publisher Full Text](#)
116. Holehouse AS, Pappu RV: **Functional Implications of Intracellular Phase**

- Transitions. *Biochemistry*. 2018; 57(17): 2415–23.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
117. **F** Brangwynne CP, Eckmann CR, Courson DS, *et al.*: **Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science*. 2009; 324(5935): 1729–32.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 118. **F** Brangwynne CP, Mitchison TJ, Hyman AA: **Active liquid-like behavior of nucleoli determines their size and shape in *Xenopus laevis* oocytes. *Proc Natl Acad Sci U S A*. 2011; 108(11): 4334–9.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 119. **F** Wippich F, Bodenmiller B, Trajkovska MG, *et al.*: **Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. *Cell*. 2013; 152(4): 791–805.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 120. Darling AL, Liu Y, Oldfield CJ, *et al.*: **Intrinsically Disordered Proteome of Human Membrane-Less Organelles. *Proteomics*. 2018; 18(5–6): e1700193.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 121. Uversky VN: **Functional roles of transiently and intrinsically disordered regions within proteins. *FEBS J*. 2015; 282(7): 1182–9.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 122. **F** Nott TJ, Petsalaki E, Farber P, *et al.*: **Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol Cell*. 2015; 57(5): 936–47.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 123. **F** Ambadipudi S, Biernat J, Riedel D, *et al.*: **Liquid–liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat Commun*. 2017; 8(1): 275.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 124. **F** Ambadipudi S, Reddy JG, Biernat J, *et al.*: **Residue-specific identification of phase separation hot spots of Alzheimer’s-related protein tau. *Chem Sci*. 2019; 10(26): 6503–7.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 125. **F** Reichheld SE, Muiznieks LD, Keeley FW, *et al.*: **Direct observation of structure and dynamics during phase separation of an elastomeric protein. *Proc Natl Acad Sci U S A*. 2017; 114(22): E4408–E4415.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 126. **F** Murthy AC, Dignon GL, Kan Y, *et al.*: **Molecular interactions underlying liquid-liquid phase separation of the FUS low-complexity domain. *Nat Struct Mol Biol*. 2019; 26(7): 637–48.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 127. **F** Ryan VH, Dignon GL, Zerze GH, *et al.*: **Mechanistic View of hnRNP2 Low-Complexity Domain Structure, Interactions, and Phase Separation Altered by Mutation and Arginine Methylation. *Mol Cell*. 2018; 69(3): 465–479.e7.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 128. **F** Brady JP, Farber PJ, Sekhar A, *et al.*: **Structural and hydrodynamic properties of an intrinsically disordered region of a germ cell-specific protein on phase separation. *Proc Natl Acad Sci U S A*. 2017; 114(39): E8194–E8203.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 129. **F** Mitrea DM, Chandra B, Ferrolino MC, *et al.*: **Methods for Physical Characterization of Phase-Separated Bodies and Membrane-less Organelles. *J Mol Biol*. 2018; 430(23): 4773–805.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 130. **F** Nasir I, Onuchic PL, Labra SR, *et al.*: **Single-molecule fluorescence studies of intrinsically disordered proteins and liquid phase separation. *Biochim Biophys Acta Proteins Proteom*. 2019; 1867(10): 980–7.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 131. **F** Taylor NO, Wei MT, Stone HA, *et al.*: **Quantifying Dynamics in Phase-Separated Condensates Using Fluorescence Recovery after Photobleaching. *Biophys J*. 2019; 117(7): 1285–300.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 132. Schwillie P, Meyer-Almes FJ, Rigler R: **Dual-color fluorescence cross-correlation spectroscopy for multicomponent diffusional analysis in solution. *Biophys J*. 1997; 72(4): 1878–86.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 133. Handwerger KE, Cordero JA, Gall JG: **Cajal bodies, nucleoli, and speckles in the *Xenopus* oocyte nucleus have a low-density, sponge-like structure. *Mol Biol Cell*. 2005; 16(1): 202–11.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 134. **F** Sokolova E, Spruijt E, Hansen MM, *et al.*: **Enhanced transcription rates in membrane-free protocells formed by coacervation of cell lysate. *Proc Natl Acad Sci U S A*. 2013; 110(29): 11692–7.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 135. **F** Strulson CA, Molden RC, Keating CD, *et al.*: **RNA catalysis through compartmentalization. *Nat Chem*. 2012; 4(11): 941–6.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 136. Nikolic J, Lagaudrière-Gesbert C, Scrima N, *et al.*: **Structure and Function of Negri Bodies. *Adv Exp Med Biol*. 2019; 1215: 111–27.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 137. Formicola N, Vijayakumar J, Besse F: **Neuronal ribonucleoprotein granules: Dynamic sensors of localized signals. *Traffic*. 2019; 20(9): 639–649.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 138. Darling AL, Zaslavsky BY, Uversky VN: **Intrinsic Disorder-Based Emergence in Cellular Biology: Physiological and Pathological Liquid-Liquid Phase Transitions in Cells. *Polymers (Basel)*. 2019; 11(6): pii: E990.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 139. Turoverov KK, Kuznetsova IM, Fonin AV, *et al.*: **Stochasticity of Biological Soft Matter: Emerging Concepts in Intrinsically Disordered Proteins and Biological Phase Separation. *Trends Biochem Sci*. 2019; 44(8): 716–28.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 140. Uversky VN: **Intrinsically Disordered Proteins and Their “Mysterious” (Meta)Physics. *Front Phys*. 2019; 7: 2985.**
[Publisher Full Text](#)
 141. Raskin DM, de Boer PA: **Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1999; 96(9): 4971–6.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 142. Lutkenhaus J: **Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu Rev Biochem*. 2007; 76: 539–62.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 143. Loose M, Fischer-Friedrich E, Herold C, *et al.*: **Min protein patterns emerge from rapid rebinding and membrane interaction of MinE. *Nat Struct Mol Biol*. 2011; 18(5): 577–83.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 144. Loose M, Kruse K, Schwillie P: **Protein self-organization: lessons from the min system. *Annu Rev Biophys*. 2011; 40: 315–36.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 145. Meinhardt H, de Boer PA: **Pattern formation in *Escherichia coli*: a model for the pole-to-pole oscillations of Min proteins and the localization of the division site. *Proc Natl Acad Sci U S A*. 2001; 98(25): 14202–7.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 146. Howard M, Rutenberg AD, de Vet S: **Dynamic compartmentalization of bacteria: accurate division in *E. coli*. *Phys Rev Lett*. 2001; 87(27 Pt 1): 278102.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 147. Kruse K: **A dynamic model for determining the middle of *Escherichia coli*. *Biophys J*. 2002; 82(2): 618–27.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 148. **F** Loose M, Fischer-Friedrich E, Ries J, *et al.*: **Spatial regulators for bacterial cell division self-organize into surface waves in vitro. *Science*. 2008; 320(5877): 789–92.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 149. **F** Zieske K, Schwillie P: **Reconstitution of self-organizing protein gradients as spatial cues in cell-free systems. *eLife*. 2014; 3: e03949.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 150. Vecchiarelli AG, Li M, Mizuuchi M, *et al.*: **Membrane-bound MinD complex acts as a toggle switch that drives Min oscillation coupled to cytoplasmic depletion of MinD. *Proc Natl Acad Sci U S A*. 2016; 113(11): E1479–88.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 151. Lagache T, Sauvonnnet N, Danglot L, *et al.*: **Statistical analysis of molecule colocalization in bioimaging. *Cytometry A*. 2015; 87(6): 568–79.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 152. Manders EMM, Verbeek FJ, Aten JA: **Measurement of co-localization of objects in dual-colour confocal images. *J Microsc*. 1993; 169(3): 375–82.**
[Publisher Full Text](#)
 153. **F** Betzig E, Patterson GH, Sougrat R, *et al.*: **Imaging intracellular fluorescent proteins at nanometer resolution. *Science*. 2006; 313(5793): 1642–5.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 154. **F** Rust MJ, Bates M, Zhuang X: **Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat Meth*. 2006; 3(10): 793–5.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 155. Louche A, Salcedo SP, Bigot S: **Protein-Protein Interactions: Pull-Down Assays. *Methods Mol Biol*. 2017; 1615: 247–55.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 156. Lee C: **Coimmunoprecipitation assay. *Methods Mol Biol*. 2007; 362: 401–6.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 157. **F** Lapetina S, Gil-Henn H: **A guide to simple, direct, and quantitative in vitro binding assays. *J Biol Methods*. 2017; 4(1): e62.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 158. Häupl B, Ihling CH, Sinz A: **Combining affinity enrichment, cross-linking with photo amino acids, and mass spectrometry for probing protein kinase D2 interactions. *Proteomics*. 2017; 17(10): e1600459.**
[PubMed Abstract](#) | [Publisher Full Text](#)
 159. Pham ND, Parker RB, Kohler JJ: **Photocrosslinking approaches to interactome mapping. *Curr Opin Chem Biol*. 2013; 17(1): 90–101.**
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 160. Brymora A, Valova VA, Robinson PJ: **Protein-Protein Interactions Identified by Pull-Down Experiments and Mass Spectrometry. *Curr Protoc Cell Biol*. 2004; 22(1): 17.5.1–17.5.51.**
[PubMed Abstract](#) | [Publisher Full Text](#)

161. **F** Fux A, Korotkov VS, Schneider M, *et al.*: **Chemical Cross-Linking Enables Drafting ClpXP Proximity Maps and Taking Snapshots of In Situ Interaction Networks.** *Cell Chem Biol.* 2019; **26**(1): 48–59.e7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
162. Kloeet SL, Baymaz HI, Makowski M, *et al.*: **Towards elucidating the stability, dynamics and architecture of the nucleosome remodeling and deacetylase complex by using quantitative interaction proteomics.** *FEBS J.* 2015; **282**(9): 1774–85.
[PubMed Abstract](#) | [Publisher Full Text](#)
163. Tanaka Y, Bond MR, Kohler JJ: **Photocrosslinkers illuminate interactions in living cells.** *Mol Biosyst.* 2008; **4**(6): 473–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
164. Sinz A: **Investigation of protein-protein interactions in living cells by chemical crosslinking and mass spectrometry.** *Anal Bioanal Chem.* 2010; **397**(8): 3433–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
165. Yang Y, Song H, Chen PR: **Genetically encoded photocrosslinkers for identifying and mapping protein-protein interactions in living cells.** *IUBMB Life.* 2016; **68**(11): 879–86.
[PubMed Abstract](#) | [Publisher Full Text](#)
166. Fields S, Song O: **A novel genetic system to detect protein-protein interactions.** *Nature.* 1989; **340**(6230): 245–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
167. **F** Rual JF, Venkatesan K, Hao T, *et al.*: **Towards a proteome-scale map of the human protein-protein interaction network.** *Nature.* 2005; **437**(7062): 1173–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
168. Brückner A, Polge C, Lentze N, *et al.*: **Yeast two-hybrid, a powerful tool for systems biology.** *Int J Mol Sci.* 2009; **10**(6): 2763–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
169. **F** Cobbert JD, DeMott C, Majumder S, *et al.*: **Caught in action: selecting peptide aptamers against intrinsically disordered proteins in live cells.** *Sci Rep.* 2015; **5**: 9402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
170. **F** Contreras-Martos S, Piai A, Kosol S, *et al.*: **Linking functions: an additional role for an intrinsically disordered linker domain in the transcriptional coactivator CBP.** *Sci Rep.* 2017; **7**(1): 4676.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
171. **F** Walter J, Charon J, Hu Y, *et al.*: **Comparative analysis of mutational robustness of the intrinsically disordered viral protein VPg and of its interactor eIF4E.** *PLoS One.* 2019; **14**(2): e0211725.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
172. Kerppola TK: **Design and implementation of bimolecular fluorescence complementation (BiFC) assays for the visualization of protein interactions in living cells.** *Nat Protoc.* 2006; **1**(3): 1278–86.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
173. Morell M, Espargaró A, Avilés FX, *et al.*: **Detection of transient protein-protein interactions by bimolecular fluorescence complementation: the Abl-SH3 case.** *Proteomics.* 2007; **7**(7): 1023–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
174. Hernández-Sánchez IE, Maruri-López I, Graether SP, *et al.*: **In vivo evidence for homo- and heterodimeric interactions of *Arabidopsis thaliana* dehydrins AtCOR47, AtERD10, and AtRAB18.** *Sci Rep.* 2017; **7**(1): 17036.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
175. Perrella G, Carr C, Asensi-Fabado MA, *et al.*: **The Histone Deacetylase Complex 1 Protein of *Arabidopsis* Has the Capacity to Interact with Multiple Proteins Including Histone 3-Binding Proteins and Histone 1 Variants.** *Plant Physiol.* 2016; **171**(1): 62–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
176. Kim DI, Roux KJ: **Filling the Void: Proximity-Based Labeling of Proteins in Living Cells.** *Trends Cell Biol.* 2016; **26**(11): 804–17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
177. Varnaité R, MacNeill SA: **Meet the neighbors: Mapping local protein interactomes by proximity-dependent labeling with BioID.** *Proteomics.* 2016; **16**(19): 2503–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
178. Rees JS, Li XW, Perrett S, *et al.*: **Protein Neighbors and Proximity Proteomics.** *Mol Cell Proteomics.* 2015; **14**(11): 2848–56.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
179. **F** Roux KJ, Kim DI, Raida M, *et al.*: **A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells.** *J Cell Biol.* 2012; **196**(6): 801–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
180. Li P, Meng Y, Wang L, *et al.*: **BioID: A Proximity-Dependent Labeling Approach in Proteomics Study.** *Methods Mol Biol.* 2019; **1871**: 143–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
181. Minde DP, Ramakrishna M, Lilley KS: **Biotin proximity tagging favours unfolded proteins and enables the study of intrinsically disordered regions.** *Commun Biol.* 2020; **3**(1): 38.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
182. Minamihata K, Goto M, Kamiya N: **Protein Heteroconjugation by the Peroxidase-Catalyzed Tyrosine Coupling Reaction.** *Bioconjug Chem.* 2011; **22**(11): 2332–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
183. Spolar RS, Record MT Jr: **Coupling of local folding to site-specific binding of proteins to DNA.** *Science.* 1994; **263**(5148): 777–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
184. Schulz GE: **Nucleotide Binding Proteins.** In: M. Balaban (Ed.) *Molecular Mechanism of Biological Recognition*, Elsevier/North-Holland Biomedical Press, Place Published. 1979; 79–94
185. Pontius BW: **Close encounters: Why unstructured, polymeric domains can increase rates of specific macromolecular association.** *Trends Biochem Sci.* 1993; **18**(5): 181–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
186. Plaxco KW, Gross M: **Cell biology. The importance of being unfolded.** *Nature.* 1997; **386**(6626): 657–659.
[PubMed Abstract](#) | [Publisher Full Text](#)
187. **F** Iakoucheva LM, Brown CJ, Lawson JD, *et al.*: **Intrinsic Disorder in Cell-signaling and Cancer-associated Proteins.** *J Mol Biol.* 2002; **323**(3): 573–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
188. Oldfield CJ, Cheng Y, Cortese MS, *et al.*: **Coupled folding and binding with alpha-helix-forming molecular recognition elements.** *Biochemistry.* 2005; **44**(37): 12454–70.
[PubMed Abstract](#) | [Publisher Full Text](#)
189. Uversky VN, Oldfield CJ, Dunker AK: **Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling.** *J Mol Recognit.* 2005; **18**(5): 343–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
190. Landsteiner K: **The Specificity of Serological Reactions.** Courier Dover Publications, Place Published. 1936.
[Publisher Full Text](#)
191. Pauling L: **A Theory of the Structure and Process of Formation of Antibodies.** *J Am Chem Soc.* 1940; **62**(10): 2643–57.
192. Karush F: **Heterogeneity of the Binding Sites of Bovine Serum Albumin.** *J Am Chem Soc.* 1950; **72**(6): 2705–13.
[Publisher Full Text](#)
193. **F** Meador WE, Means AR, Quirocho FA: **Modulation of calmodulin plasticity in molecular recognition on the basis of x-ray structures.** *Science.* 1993; **262**(5140): 1718–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
194. Kriwacki RW, Hengst L, Tennant L, *et al.*: **Structural studies of p21Waf1/Cip1/Sd1 in the free and Cdk2-bound state: conformational disorder mediates binding diversity.** *Proc Natl Acad Sci U S A.* 1996; **93**(21): 11504–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
195. Uversky VN: **Protein folding revisited. A polypeptide chain at the folding-misfolding-nonfolding cross-roads: which way to go?** *Cell Mol Life Sci.* 2003; **60**(9): 1852–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
196. Dunker AK, Garner E, Guilloit S, *et al.*: **Protein disorder and the evolution of molecular recognition: Theory, predictions and observations.** *Pac Symp Biocomput.* 1998; 473–84.
[PubMed Abstract](#)
197. Oldfield CJ, Meng J, Yang JY, *et al.*: **Flexible nets: Disorder and induced fit in the associations of p53 and 14-3-3 with their partners.** *BMC Genomics.* 2008; **9** Suppl 1: S1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
198. Bourhis JM, Johansson K, Receveur-Bréchet V, *et al.*: **The C-terminal domain of measles virus nucleoprotein belongs to the class of intrinsically disordered proteins that fold upon binding to their physiological partner.** *Virus Res.* 2004; **99**(2): 157–67.
[PubMed Abstract](#) | [Publisher Full Text](#)
199. Mohan A, Oldfield CJ, Radivojac P, *et al.*: **Analysis of Molecular Recognition Features (MoRFs).** *J Mol Biol.* 2006; **362**(5): 1043–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
200. Cheng Y, Oldfield CJ, Meng J, *et al.*: **Mining alpha-helix-forming molecular recognition features with cross species sequence alignments.** *Biochemistry.* 2007; **46**(47): 13468–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
201. Vacic V, Oldfield CJ, Mohan A, *et al.*: **Characterization of molecular recognition features, MoRFs, and their binding partners.** *J Proteome Res.* 2007; **6**(6): 2351–66.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
202. Meng F, Uversky VN, Kurgan L: **Comprehensive review of methods for prediction of intrinsic disorder and its molecular functions.** *Cell Mol Life Sci.* 2017; **74**(17): 3069–90.
[PubMed Abstract](#) | [Publisher Full Text](#)
203. Disfani FM, Hsu WL, Mizianty MJ, *et al.*: **MoRFPred, a computational tool for sequence-based prediction and characterization of short disorder-to-order transitioning binding regions in proteins.** *Bioinformatics.* 2012; **28**(12): i75–83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
204. Fang C, Noguchi T, Tominaga D, *et al.*: **MFSPPSMPred: identifying short disorder-to-order binding regions in disordered proteins based on contextual local evolutionary conservation.** *BMC Bioinformatics.* 2013; **14**: 300.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
205. Malhis N, Gsponer J: **Computational identification of MoRFs in protein sequences.** *Bioinformatics.* 2015; **31**(11): 1738–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

206. Malhis N, Jacobson M, Gsponer J: **MoRFchibi SYSTEM: software tools for the identification of MoRFs in protein sequences.** *Nucleic Acids Res.* 2016; **44**(W1): W488–W493.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
207. Yan J, Dunker AK, Uversky VN, *et al.*: **Molecular recognition features (MoRFs) in three domains of life.** *Mol BioSyst.* 2016; **12**(3): 697–710.
[PubMed Abstract](#) | [Publisher Full Text](#)
208. Xue B, Dunker AK, Uversky VN: **Retro-MoRFs: identifying protein binding sites by normal and reverse alignment and intrinsic disorder prediction.** *Int J Mol Sci.* 2010; **11**(10): 3725–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
209. **F** Jones DT, Cozzetto D: **DISOPRED3: precise disordered region predictions with annotated protein-binding activity.** *Bioinformatics.* 2015; **31**(6): 857–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
210. Khan W, Duffy F, Pollastri G, *et al.*: **Predicting binding within disordered protein regions to structurally characterised peptide-binding domains.** *PLoS One.* 2013; **8**(9): e72838.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
211. Mészáros B, Simon I, Dosztányi Z, *et al.*: **Prediction of protein binding regions in disordered proteins.** *PLoS Comput Biol.* 2009; **5**(5): e1000376.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
212. Dosztányi Z, Mészáros B, Simon I: **ANCHOR: web server for predicting protein binding regions in disordered proteins.** *Bioinformatics.* 2009; **25**(20): 2745–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
213. Peng Z, Kurgan L: **High-throughput prediction of RNA, DNA and protein binding regions mediated by intrinsic disorder.** *Nucleic Acids Res.* 2015; **43**(18): e121–e121.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
214. **F** Mooney C, Pollastri G, Shields DC, *et al.*: **Prediction of short linear protein binding regions.** *J Mol Biol.* 2012; **415**(1): 193–204.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
215. Meng F, Kurgan L: **DfLpred: High-throughput prediction of disordered flexible linker regions in protein sequences.** *Bioinformatics.* 2016; **32**(12): i341–i350.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
216. **F** Zarin T, Strome B, Nguyen Ba AN, *et al.*: **Proteome-wide signatures of function in highly diverged intrinsically disordered regions.** *eLife.* 2019; **8**: pii: e46883.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
217. Iakoucheva LM, Radivojac P, Brown CJ, *et al.*: **The importance of intrinsic disorder for protein phosphorylation.** *Nucleic Acids Res.* 2004; **32**(3): 1037–49.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
218. Pejaver V, Hsu WL, Xin F, *et al.*: **The structural and functional signatures of proteins that undergo multiple events of post-translational modification.** *Protein Sci.* 2014; **23**(8): 1077–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
219. Daily KM, Radivojac P, Dunker AK: **Intrinsic disorder and protein modifications: building an SVM predictor for methylation.** *IEEE Symposium on Computational Intelligence in Bioinformatics and Computational Biology, CIBCB, San Diego, California, U.S.A. 2005; 475–481.*
[Reference Source](#)
220. Radivojac P, Vacic V, Haynes C, *et al.*: **Identification, analysis, and prediction of protein ubiquitination sites.** *Proteins.* 2010; **78**(2): 365–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
221. Reddy KD, Malipeddi J, DeForte S, *et al.*: **Physicochemical sequence characteristics that influence S-palmitoylation propensity.** *J Biomol Struct Dyn.* 2016; **35**(11): 2337–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
222. **F** Oates ME, Romero P, Ishida T, *et al.*: **D²P²: database of disordered protein predictions.** *Nucleic Acids Res.* 2013; **41**(Database issue): D508–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
223. Gouw M, Sámano-Sánchez H, Van Roey K, *et al.*: **Exploring Short Linear Motifs Using the ELM Database and Tools.** *Curr Protoc Bioinformatics.* 2017; **58**: 8.22.1–8.22.35.
[PubMed Abstract](#) | [Publisher Full Text](#)
224. Gouw M, Michael S, Sámano-Sánchez H, *et al.*: **The eukaryotic linear motif resource - 2018 update.** *Nucleic Acids Res.* 2018; **46**(D1): D428–D434.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
225. **F** Vernon RM, Forman-Kay JD: **First-generation predictors of biological protein phase separation.** *Curr Opin Struct Biol.* 2019; **58**: 88–96.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
226. Lancaster AK, Nutter-Upham A, Lindquist S, *et al.*: **PLAAC: a web and command-line application to identify proteins with prion-like amino acid composition.** *Bioinformatics.* 2014; **30**(17): 2501–2.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
227. **F** Alberti S, Halfmann R, King O, *et al.*: **A systematic survey identifies prions and illuminates sequence features of prionogenic proteins.** *Cell.* 2009; **137**(1): 146–58.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
228. **F** Vernon RM, Chong PA, Tsang B, *et al.*: **Pi-Pi contacts are an overlooked protein feature relevant to phase separation.** *eLife.* 2018; **7**: pii: e31486.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
229. Hughes MP, Sawaya MR, Boyer DR, *et al.*: **Atomic structures of low-complexity protein segments reveal kinked β sheets that assemble networks.** *Science.* 2018; **359**(6376): 698–701.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
230. Wang J, Choi JM, Holehouse AS, *et al.*: **A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins.** *Cell.* 2018; **174**(3): 688–699.e16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
231. Bolognesi B, Lorenzo Gotor N, Dhar R, *et al.*: **A Concentration-Dependent Liquid Phase Separation Can Cause Toxicity upon Increased Protein Expression.** *Cell Rep.* 2016; **16**(1): 222–31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
232. **F** Orlando G, Raimondi D, Tabaro F, *et al.*: **Computational identification of prion-like RNA-binding proteins that form liquid phase-separated condensates.** *Bioinformatics.* 2019; **35**(22): 4617–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
233. Sun T, Li Q, Xu Y, *et al.*: **Prediction of liquid-liquid phase separation proteins using machine learning.** *bioRxiv.* 2019.
[Publisher Full Text](#)
234. **F** Li Q, Peng X, Li Y, *et al.*: **LLPSDB: a database of proteins undergoing liquid-liquid phase separation in vitro.** *Nucleic Acids Res.* 2020; **48**(D1): D320–D327.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
235. DeForte S, Uversky VN: **Order, Disorder, and Everything in Between.** *Molecules.* 2016; **21**(8): pii: E1090.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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