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REVIEW

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Features of molecular recognition of intrinsically disordered proteins via coupled folding and binding

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Abstract

The sequence–structure–function paradigm of proteins has been revolutionized by the discovery of intrinsically disordered proteins (IDPs) or intrinsically disordered regions (IDRs). In contrast to traditional ordered proteins, IDPs/IDRs are unstructured under physiological conditions. The absence of well-defined three-dimensional structures in the free state of IDPs/IDRs is fundamental to their function. Folding upon binding is an important mode of molecular recognition for IDPs/IDRs. While great efforts have been devoted to investigating the complex structures and binding kinetics and affinities, our knowledge on the binding mechanisms of IDPs/IDRs remains very limited. Here, we review recent advances on the binding mechanisms of IDPs/IDRs. The structures and kinetic parameters of IDPs/IDRs can vary greatly, and the binding mechanisms can be highly dependent on the structural properties of IDPs/IDRs. IDPs/IDRs can employ various combinations of conformational selection and induced fit in a binding process, which can be templated by the target and/or encoded by the IDP/IDR. Further studies should provide deeper insights into the molecular recognition of IDPs/IDRs and enable the rational design of IDP/IDR binding mechanisms in the future.

KEYWORDS

binding kinetics, fuzzy interaction, intrinsically disordered proteins, molecular recognition, transition state

1 | INTRODUCTION

Proteins are important biological molecules. The three-dimensional (3D) structure, which is determined by the primary amino-acid sequence, is critical for a protein to carry out its functions. Traditionally, proteins are classified as being either ordered (folded) or disordered (unfolded) by analyzing their conformational states. Ordered proteins have well-defined 3D structures and exhibit small-scale structural fluctuations under physiological conditions. On the contrary, intrinsically disordered proteins could sample an ensemble of conformations which may be compact (molten globule-like) or extended (coil-like or pre-molten globule-like).[1–5] Furthermore, proteins can be entirely disordered polypeptides (IDPs) or a combination of disordered regions (IDRs) and ordered domains.[6–8]

Based on bioinformatics predictions, IDPs/IDRs are abundant in all species.[9–11] By analyzing the proteomes of 3,484 species and correlating the fraction of disordered residues with proteome size, it is shown that eukaryotes have more disordered residues than prokaryotes.[12] A recent comprehensive analysis of over 6 million proteins characterized intrinsic disorder at proteomic and protein levels indicates that IDPs/IDRs

Abbreviations: 3D, three-dimensional; AS, alternative splicing; IDPs, intrinsically disordered proteins; IDRs, intrinsically disordered regions; LFER, linear free-energy relationships; LLPS, liquid–liquid phase separation; MoRFs, molecular recognition features; PTM, posttranslational modification; SLiMs, short linear motifs.

Jing Yang and Meng Gao contributed equally to this study.

are more abundant in eukaryotes and certain functions are exclusively implemented by IDPs/IDRs.[13] The correlation between the organism complexity and the amount of intrinsic disorder are consistent with the extensive involvement of IDPs/IDRs in regulatory and signaling functions and the increased disorder content in eukaryotic proteomes might be used by nature to deal with the increased cellular complexity.[12,14,15]

IDPs/IDRs are involved in various biological functions. In a comprehensive bioinformatics study carried out by Xie et al.,[16,17] a positive correlation between the functional annotation of the SwissProt database and the predicted intrinsic disorder has been found. Generally, IDPs/IDRs are enriched in proteins involved in signaling and regulatory functions, including transcription regulation, cell cycle, mRNA processing, scaffolding, and apoptosis.[6,14,15,18–31] Consequently, dysregulation of IDPs/IDRs are associated with a variety of human diseases.[32–44] Most recently, many IDPs/IDRs are found to be able to undergo liquid–liquid phase separation (LLPS), which is related to the assembling of membraneless organelles in vivo.[45–53] So far, studies on IDPs/IDRs have greatly extended our understanding on the sequence–structure–function relationship of proteins.[29,54–56] Recently, the protein structure–function continuum concept was proposed by Uversky to illustrate the numerous biological functions of p53 through multiple proteoforms by various mechanisms and may be extended to many multi-function IDPs.[57]

In this review, we will summarize recent advances of our understanding on the molecular recognition of IDPs/IDRs. We will focus on specific interactions between IDPs/IDRs and their targets, which usually result in folding of the IDPs/IDRs upon target binding. We will discuss the mechanistic features of molecular recognition inferred from kinetics, thermodynamics, and structure investigations.

2 | MOLECULAR RECOGNITION FEATURES

The flexible structures of IDPs/IDRs make them suitable for cellular regulatory and dynamic signaling processes.[14] Several functional modes have been summarized for IDPs/IDRs, including entropic chains, effectors, scavengers, assemblers, display sites, and chaperones.[7,30,58] A common module for molecular recognition within IDPs/IDRs is often known as molecular recognition features (MoRFs) or short linear motifs.[59–63] The sequence features of MoRFs are distinct from the rest portion of IDPs/IDRs, enabling development of predictors to identify MoRFs.[64] For example, ANCHOR predicts disordered binding regions based on the pairwise energy estimation from IUPred.[65–68] Usually, upon binding to their partners, MoRFs undergo disorder-to-order transitions. This process is termed coupled folding-binding.[69,70] The structures of MoRFs adopted upon binding can be divided into three types: α-helix, β-strand, and irregular secondary structure. IDPs/IDRs can utilize multiple MoRFs simultaneously when interacting with their binding partners (Figure 1).[59]

Studying the recognition mechanisms of IDPs/IDRs with their partners is not a trivial task. In recent years,

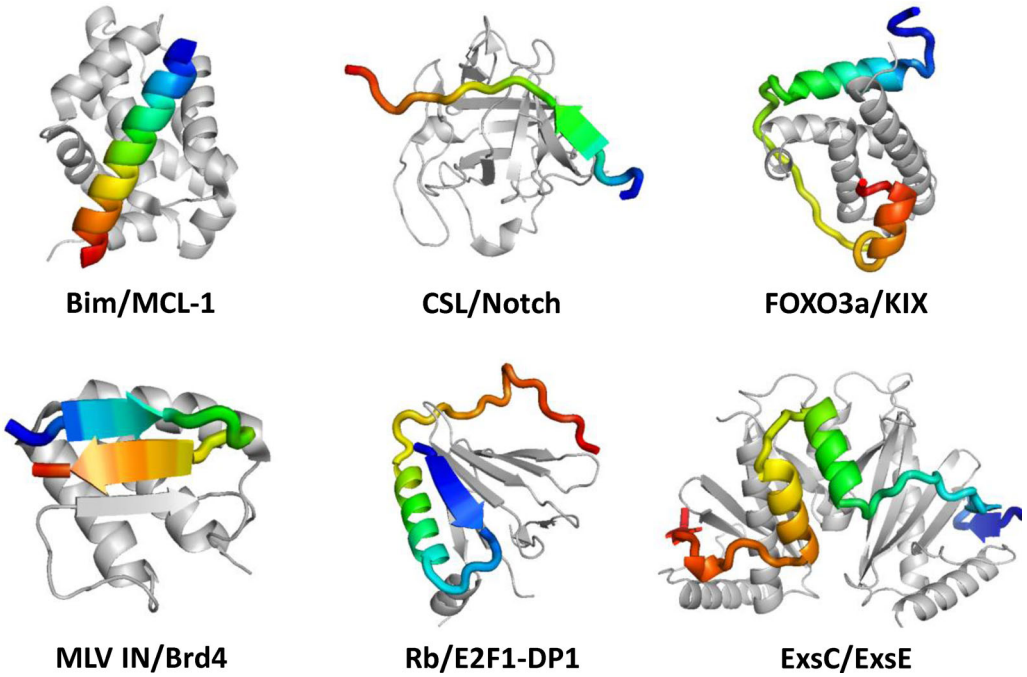


FIGURE 1 Examples of intrinsically disordered protein (IDP) complex with various combinations of molecular recognition features (MoRFs). IDPs are shown in rainbow color and the targets are shown in gray. PDB IDs are: Bim/MCL-1 (2NL9), CSL/notch (2FO1), FOXO3a/KIX (2LQI), MLV IN/Brd4 (2N3K), Rb/E2F1-DP1 (2AZE), and ExsC/ExsE (3KXY)

however, large progress has been made from a close collaboration between experimental and computational studies. A spectrum of techniques have been applied to study IDPs/IDRs, providing valuable information on their structures, dynamic properties and binding mechanisms.[2,3,71–75] In parallel, molecular modeling and computer simulations provide atomic pictures of conformation ensembles and binding processes as well as reveal important underlying physical principles.[76–82]

3 | RATE CONSTANTS

Coupled folding with binding has been suggested to enhance the binding rates of IDPs/IDRs.[83] Theoretical analysis and computer simulations predicted that the "fly-casting" effect accelerates the binding rate by twofolds to threefolds.[83–85] Consistent with this prediction, rate constants of IDP/IDR-protein interactions from the literature show differences from those of ordered proteins in a general trend.[85] The binding kinetics of IDPs/IDRs is affected not only by the overall structure flexibility, but also by the local conformation preference of MoRFs. Stabilizing the preformed conformation of MoRFs has been found to accelerate the association rate constants (k_{on}), due to an increase of the probability of converting collision complexes to bound state.[86–90] On the other hand, increasing the degree of disorder has been found to significantly increase the dissociation rate constant (k_{off}), suggesting that the dominant effect of disorder on molecular recognition may be to accelerate dissociation rather than association.[87,91] While computer simulations provide detailed correlation between conformation disorder and binding/unbinding rate constants, it is difficult to test the actual role of disorder in binding kinetics experimentally as it is hard to quantify the extent of disorder and the influence on binding kinetics could be resulted from changes in the interactions between the target and IDP/IDR or changes in the binding mechanism.

Electrostatic interactions could play important roles in the coupled folding-binding process of IDPs/IDRs as many MoRFs contain charged residues.[92] For many studied IDPs/IDRs, the k_{on} values are reduced for about ten folds when the salt concentration increases from low (~50 mM) to high (~500 mM), that is, $\partial\log(k_{on})/\partial\log(C_{salt}) \approx -1$ (Table 1), indicating the presence of favorable electrostatic interactions. Molecular dynamics simulations found that long-range electrostatic interactions accelerate the binding rate in a range consistent with experimental results.[97,104–108] More importantly, simulations revealed that electrostatic forces enhance the binding kinetics not only by increasing the encounter rate but also by enhancing the efficiency of IDPs/IDRs evolving to the bound states upon encounter.[105,108] Advances in single molecule techniques allow the detection of transient species during a coupled folding-binding process. Interestingly, the transition path times of ACTR/NCBD interaction is much longer than the transition path times of protein folding, indicating the presence of stable intermediate state along the binding

TABLE 1 Effect of salt concentration on the association rate constant of IDPs

IDP	Number of charges		Target	$\partial\log(k_{on})/\partial\log(C_{salt})$	Reference
$p53_{13\text{-}61}$	−11	+1	NCBD	−2.10	93
HPV E7	−4	+0	Rb	−1.56	94
MLL	−6	+1	KIX	−1.52	95
STAT2	−10	+4	TAZ1	−1.32	96
ACTR	−11	+4	NCBD	−1.26	93
p27	−16	+14	Cdk2/cyclin A	−1.21	97
SRC1	−11	+5	NCBD	−1.15	93
$E3^{IDP}$	−12	+20	Im3	−1.06	98
TIF2	−9	+2	NCBD	−0.86	93
PUMA	−10	+6	MCL-1	−0.68	99
WASP	−21	+21	Cdc42	−0.62	100
$p53_{13\text{-}63}$	−12	+1	Mdm2	−0.56	101
$p73_{11\text{-}25}$	−3	+0	Mdm2	−0.38	101
c-Myb	−5	+5	KIX	−0.25	102
$p53_{15\text{-}29}$	−3	+1	Mdm2	−0.21	101
$p63_{52\text{-}65}$	−3	+0	Mdm2	0.13	101
eIF4G	−2	+4	eIF4E	0.75	103

Abbreviation: IDP, intrinsically disordered protein.

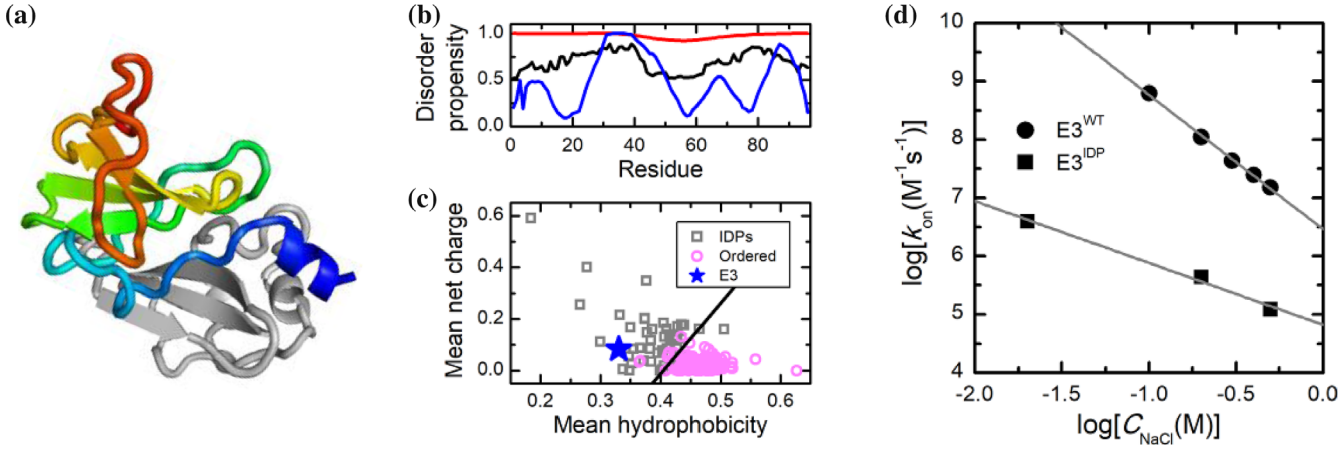


FIGURE 2 Binding of E3 to Im3. (a) Crystal structure of E3/Im3 complex. E3 is shown in rainbow color and Im3 in gray. (b) Disorder propensity prediction of E3 using three different predictors: IUPred2 (black), MFDp2 (red), and PONDR VL-XT (blue). (c) Location of E3 on the charge-hydrophobicity plot. The black line indicates the boundary between the intrinsically disordered proteins (IDPs) region and the ordered proteins. (d) Effect of salt concentration on k_{on} for $E3^{WT}$ and $E3^{IDP98}$

process.[109] Furthermore, the lifetime of transient complexes for ACTR/NCBD is also longer than that for barnase/barstar,[110] consistent with previous simulation predictions.[85]

It is noted that as the conformations of IDPs/IDRs are highly dynamic, electrostatic interactions between IDPs/IDRs and their targets during the encounter process may be different from those of the corresponding ordered proteins, which may be reflected from a recent study on the interactions between the colicin E3 rRNase domain (E3) and the immunity protein Im3 (Figure 2a).[98] Although E3 is a folded domain, disorder prediction suggests that it contains high disorder propensities (Figure 2b,c). Actually, a single alanine mutation at Tyr507 within the hydrophobic core of E3 causes the protein to become an IDP ($E3^{IDP}$). Kinetics studies show that k_{on} of $E3^{WT}$ with Im3 is decreased by three orders of magnitude when the salt concentration is increased. However, under the same range of salt concentration, k_{on} of $E3^{IDP}$ with Im3 is only decreased by less than 40 folds (Figure 2d).[98] However, as the mechanism of $E3^{IDP}$ binding to Im3 is unclear, it is unknown whether $E3^{IDP}$ folds before binding or folds during binding. In this context, the salt dependence of the association rate constant for $E3^{IDP}$/Im3 interaction remains elusive.

4 | CONFORMATIONAL SELECTION, INDUCED FIT AND BEYOND

Recently, Dunker and Oldfield[111] suggested that the interaction between an IDP/IDR and its partner should not be described as induced fit where the protein is folded but can adjust its structure to fit the substrate. However, since the discovery of IDPs/IDRs, the sequence–structure paradigm has been revolutionized. In this context, it should be reasonable to expand the concepts of induced fit to analyze the binding processes of IDPs/IDRs. Thus, in an induced fit process, unfolded conformations of an IDP/IDR are able to weakly interact with the target to form encounter complexes which induce the unfolded conformations fold into the bound conformations. On the contrary, in a conformational selection process, an IDP/IDR samples unfolded conformation as well as pre-folded conformations and only the prefolded conformations are binding competent.

Conformational selection and induced fit have been widely applied to explain the coupled folding-binding process of IDPs/IDRs.[75,112,113] Which mechanism dominates during the binding process depends on several factors, including the structure preference and conformational dynamics of the IDPs/IDRs, the association rate, and the concentration as well.[114–125] It has been established that IDPs/IDRs sample a variety of conformations rapidly.[126–129] At one extreme, if the conformation ensemble of an IDP/IDR in the unbound state is completely different from that in the bound state, it is expected that the binding process proceeds via the induced fit mechanism. Except this extreme condition, (partially) bound-like conformations could be sampled by the free IDPs/IDRs. It is plausible that these preformed bound-like conformations can also initiate the binding process. Under such circumstance, the observed binding mechanism is determined by a competition between the flux of conformational selection and that of induced fit.[119–122] The flux from unbound state to bound state is determined by the folding/unfolding rate constants, association/dissociation rate constants as well as protein concentrations.[119] The flux description predicts that conformational selection is favored when the folding kinetics of free IDPs/IDRs is fast, affinity for inactive conformations is low, and protein concentration is low.[121] In another study, similar conclusions are reached for the effect of protein concentration

via molecular dynamics simulations; however, the effect of conformation transition kinetics is opposite.[120] Sampling of the bound-like conformations in the unbound state is necessary but not sufficient for a conformational selection process. As conformational transitions occur in the unbound state as well as in the loosely bound state, increasing conformation transition kinetics will push the mechanism toward induced fit.[120,122]

Several experimental strategies have been proposed to distinguish conformational selection from induced fit. Weikl and Deuster[114] proposed a framework by perturbing the conformational equilibrium between the inactive conformations and active conformations via introducing mutation far from ligand binding site. In the case of conformational selection, such mutations will mainly change the association rate, whereas in the case of induced fit, the dissociation rate will be mainly affected. In the interactions between the BH3 motif of PUMA and the structured protein MCL-1, the helical structure of PUMA was modulated by mutating solvent-exposed residues to proline or glycine.[87,130] The mutations resulted in a modest effect on k_{on} but a significant effect on k_{off}, suggesting that the PUMA/MCL-1 interaction is an induced fit process. Stabilizing the helical conformation by trifluoroethanol may be applied to perturb the conformational equilibrium as well. Increasing the trifluoroethanol concentration increased the helix content of c-Myb and decreased the dissociation rate of c-Myb/KIX complex, suggesting that folding of c-Myb is induced after KIX binding.[131] The ACTR/NCBD interaction was investigated by selectively perturbing the amount of secondary structure in free ACTR via mutation and k_{on} and k_{off} were affected to similar extent,[86,89] suggesting that conformational selection is involved in the ACTR/NCBD binding process. While mutational analysis provides clues to speculate the binding mechanism, a correlation between helix propensity and rate constants is not a proof for conformational selection.[116,132] Other proposed strategies rely on measuring the observed rate constant under various ligand or/and target concentrations and investigating the dependence of observed rate constant on concentration.[115–118] For example, a comparison of the observed rate constant for various ACTR/NCBD concentrations and N_{TAIL}/XD concentrations suggest that their binding processes are induced fit.[116,133,134]

From studies on IDP/IDR-protein interactions, it is likely that the binding processes are induced fit combined with various degree of conformational selection.[75,135] As discussed above, the relative flux through these two pathways is determined by the protein concentrations, association rate and conformation transition kinetics. It is plausible that the partially preformed bound-like conformations play a role in the initial binding step, forming weak encounter complexes which further evolve into the bound conformation.[90] Recently, through NMR investigation, Schneider et al.[136] found that the free-state conformational equilibrium of N_{TAIL} is funneled by interactions with XD, leading to preformed bound-like conformations in the encounter complex. Thus, the free-state conformational transition of an IDP/IDR and its interactions with the target are coupled in the "conformational funneling" description of the folding-binding process.[132,136] Structure information on the encounter complexes, the intermediates, and transition states will be of great value for comprehensive understanding of the entire binding process.

5 | THE TRANSITION STATES

It is important to analyze the transition state to understand how a coupled folding-binding process crosses the free energy barrier. This can be achieved by ϕ-value analysis and linear free-energy relationships (LFERs) analysis. In a coupled-folding binding process, the ϕ-values are calculated from the free energy change for the transition state ($\Delta\Delta G^{\ddagger}$) and at equilibrium ($\Delta\Delta G_{Eq}$):

$$\phi = \frac{\Delta\Delta G^{\ddagger}}{\Delta\Delta G_{Eq}}, \tag{1}$$

$$\Delta\Delta G^{\ddagger} = RT\ln\left(\frac{k_{on}^{\text{wild-type}}}{k_{on}^{\text{mutant}}}\right), \tag{2}$$

$$\Delta\Delta G_{Eq} = RT\ln\left(\frac{K_d^{\text{mutant}}}{K_d^{\text{wild-type}}}\right). \tag{3}$$

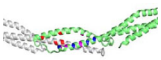
By comparing the influence of a point mutation on k_{on} and K_d, the ϕ-value of a residue provides information on the proportion of native contacts (either intermolecular or intramolecular) it makes at the transition state. In general, residues with $\phi \approx 0$ and $\phi \approx 1$ indicate their structures in the transition state resemble those in the unbound state and the bound state, respectively.

Although with a lower resolution, the location of transition state can also be inferred from the LFER analysis. Small structure alteration on the unbound molecules will result in changes in the complex stability and association kinetics by:

$$\log\frac{k_{on}^{MT}}{k_{on}^{WT}} = \alpha\log\frac{K_d^{WT}}{K_d^{MT}}. \tag{4}$$

The parameter α ($0 \le \alpha \le 1$) measures the location of the transition state along the binding path. Binding processes with $\alpha \approx 0$ and $\alpha \approx 1$ mean that the transition state is unbound-like and bound-like, respectively. The LFER analysis is helpful to identify the location of transition state when mutations result in small changes in stability, prohibiting reliable calculations of ϕ-values.

TABLE 2 ϕ-Value analysis of coupled folding-binding process

IDP	Target	ϕ-Value distribution	Reference	IDP	Target	ϕ-Value distribution	Reference
HIF-1α	TAZ1	 PDB: 1L8C	137	c-Myb	KIX	 PDB: 1SB0	88,138,139
STAT2	TAZ1	 PDB: 2KA4	96	pKID	KIX	 PDB: 1KDX	140
PUMA	A1	 PDB: 2VOF	141	MLL	KIX	 PDB: 2LXS	142
PUMA	MCL-1	 PDB: 2ROC	130,141	E6 peptide	PDZ2	 PDB: 2I0L	143
BID	A1	 PDB: 2VOI	141	S peptide	S protein	 PDB: 1D5D	144
BID	MCL-1	 PDB: 2KBW	141	N_{TAIL}	X domain	 PDB: 1T6O	145
α-Spectrin	β-Spectrin	 PDB: 3LBX	146	ACTR	NCBD	 PDB: 1KBH	86,147,148
C-terminal tail of nNOS PDZ	Syntrophin PDZ	 PDB: 1QAV	149				

Note: The IDPs are colored in light green and the targets are colored in gray. Residues with low ($\phi \leq 0.25$), medium ($0.25 < \phi < 0.6$), and high ($\phi \geq 0.6$) ϕ-values are highlighted in blue, magenta, and red, respectively.

ϕ-value analysis and LFER analysis have been applied to many IDP/IDR complexes (Table 2). In most studied cases, low fractional values of ϕ and α are commonly observed, indicating that IDPs/IDRs remain largely unstructured in the transition states. This is manifested in the conformation ensemble of transition states obtained via molecular dynamics simulations using ϕ-values as restraints.[138,147] It is noted that ϕ-values are not evenly distributed along the sequences. Residues with high ϕ-value may serve as the anchor sites to stabilize the encounter complexes, allowing the encounter complexes to cross the free energy barrier and evolve to native bound states. This picture resembles the dock-and-coalesce mechanism proposed by Zhou et al.[150] Furthermore, low values of ϕ and α highlight the importance of non-specific interactions (including electrostatic and hydrophobic interactions) in the initial stage of binding, probably stabilizing the encounter complexes.

6 | IDPS/IDRS ENCODED BINDING VERSUS TARGET TEMPLATED FOLDING

Since IDPs/IDRs are mainly unfolded in their unbound states, their folded structures observed in the complex state should be induced or stabilized by the binding partners. It remains unclear how a coupled folding-binding process is encoded. The sequence–structure relationship of proteins tells that the 3D structure of a protein is primarily encoded by its sequence. Extending this paradigm to the coupled folding-binding of IDPs/IDRs, it is expected that the folded structure of an IDP/IDR in its bound state and its binding mechanism are determined by its sequence and/or the target's sequence (thus the target's structure).

An IDP/IDR binds to diverse targets and folds into similar structures resembling its free conformation ensemble should support that the folded structure of an IDP/IDR is encoded by its sequence. An example is the N-terminal transactivation domain of p53, which forms similar α-helical structures upon binding to Mdm2 (PDB 1YCR), MdmX (PDB 2MWY), TAZ1 (PDB 5HOU), TAZ2 (PDB 2MZD), tfb1 PH domain (PDB 2GS0), RPA70 (PDB 2B3G), HMGB1 (PDB 2LY4), and NCBD (PDB 2L14). Besides the folded structure, the binding mechanism can also be encoded by the IDPs/IDRs. Clarke et al[141] compared the transition states between the disordered BH3-only proteins PUMA and BID and the folded BCL-2–like proteins A1 and MCL-1 using ϕ-value analysis. They found that the ϕ-value profiles for PUMA and BID are conserved when binding to different partners, suggesting that the binding processes of PUMA and BID are encoded by the IDPs/IDRs but not templated by the partners (Figure 3a). Recently, Wu

and Zhou altered the binding pathways of WASP with Cdc42, either suppressed the original dominant pathway or promoted a new dominant pathway through manipulating the charged residues on WASP,[151] further emphasized the role of IDPs/IDRs in encoding the binding process.

On the other hand, there are also evidences showing that the folding/binding process is templated by the target. Although they bind to the same pocket of S100B, the C-terminal segment of p53 and TRTK-12 show low sequence similarity and form different bound conformations. However, computer simulations show strong similarities in the binding intermediate states of the two peptides, suggesting that S100B templates the binding process.[152] Toto et al. perturbed the hydrophobic network of KIX using site-directed mutagenesis and investigated the folding mechanism of c-Myb with wild-type and mutated KIX.[138] By performing a LFER analysis, they found that the α-value decreases from 0.89 for wild-type KIX to around 0.5 for I26V and L43A mutants and to 0.19 for I72V mutant. The decrease in α-value appears to be correlated to the decrease in the hydrophobic solvent accessible surface area in the binding site on KIX [Figure 3b]. It is surprised to find that the structure of KIX dictates the folding mechanism of c-Myb as c-Myb has a strong propensity for α-helix formation in its N-terminus and the coupled folding-binding process of c-Myb to KIX has been suggested to involve elements of conformational selection.[88] The templated folding mechanism has been suggested to enable IDPs/IDRs to be specifically recognized by multiple targets.[138] A similar strategy was applied to investigate the interactions between N_{TAIL} and XD and revealed that the binding process of N_{TAIL} is very malleable and is affected by the structure of XD.[153] On the contrary, mutagenesis and ϕ-value analysis revealed that the transition state of ACTR/NCBD complex is highly heterogeneous and is robust with respect to most mutations for ACTR or NCBD.[147]

As IDPs/IDRs possess various sequence and structure preferences, the above discussions indicate that the coupled folding-binding process of an IDP/IDR could be templated by the partner as well as encoded by its sequence. Further mechanistic studies are required to reveal the microscopic details on how a target templates or how an IDP/IDR encodes the binding/folding process.

7 | EFFECT OF MACROMOLECULAR CROWDING

The intracellular environment is very crowded since up to 40% of the volume of a cell is occupied by biological macromolecules.[154] Macromolecular crowding can affect protein–target binding and protein folding.[155] In particular, the malleability of IDPs/IDRs makes them susceptible to the influence of macromolecular crowders.[156,157] Conformational compaction of IDPs/IDRs by macromolecular crowders has been observed, where the effect depends not only on the crowder size and concentration, but also on the properties of IDPs/IDRs.[158–163] MAP2c, $p21^{Cip1}$, and FlgM show global compaction and local structuring in crowded conditions.[164,165] The distal helix of calcineurin and transiently helical regions of ACTR are also stabilized when crowded by synthetic polymers.[166,167] However, conformational compaction induced by crowders is not necessary to promote secondary structure formation for IDPs/IDRs. For example, α-Casein, the C-terminal activation domain of c-Fos, and the kinase-inhibition domain of $p27^{Kip1}$ shows little structural changes under crowded conditions.[164,168]

Besides modulating the conformational properties of IDPs/IDRs, macromolecular crowding also affects their diffusion properties. In general the translational and rotational diffusions of IDPs/IDRs are reduced.[169,170] Interestingly, the effect of crowding on the diffusion of IDPs/IDRs is less than that on folded proteins.[169,170] Consequently, larger IDPs/IDRs may diffuse faster than smaller folded proteins in cells.[169] Recently, study on FlgM under crowded condition reveals the presence of extended conformations which snake through interstitial crevices and bind multiple crowders simultaneously.[171] It is probable that such extended conformations may facilitate recognition of IDPs/IDRs under crowded conditions.

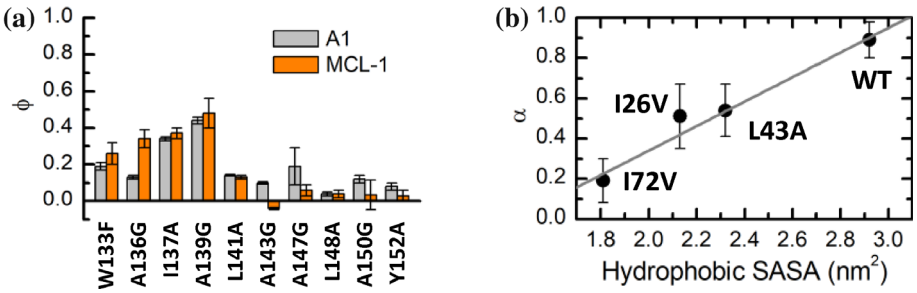


FIGURE 3 Illustrations of intrinsically disordered protein (IDP) encoded binding and target templated binding. (a) ϕ-values for the disordered BH3-only protein PUMA binding with BCL-2–like proteins A1 and MCL-1.[141] (b) correlation of α-value from linear free-energy relationships (LFER) analysis for c-Myb with the hydrophobic solvent accessible surface area in the binding site on KIX[138]

It is also important to directly study the effect of macromolecular crowding on the molecular recognition process. Binding of calmodulin with CaMKI peptide was investigated under crowded conditions.[172] It was found that the on- and off-rates are reduced by about two folds in a compensatory fashion, thus the binding affinity is almost not changed. The reduction of association rate constant suggests that binding of CaMKI peptide with calmodulin is under diffusion control and crowding slows down the diffusion process.[155,172] For reaction control binding process, it is expected that the association rate constant will be increased.[155] In another study, computer simulation on the coupled folding-binding of pKID with KIX showed that the folding-binding mechanism observed in bulk solution remains unchanged under highly crowded conditions.[173] It seems that molecular crowding has small effect on the binding mechanism of IDPs/IDRs.

8 | DYNAMIC CONTACTS AND FUZZY INTERACTIONS

While the main recognition elements are folded upon binding for many IDPs/IDRs, they may still exhibit conformational dynamics in the complex state.[174,175] For example, the TAD of STAT2 only undergoes a partial disorder-to-order transition upon binding with TAZ1 and retains subnanosecond motions.[176] Conformational dynamics in the bound state enables the IDPs/IDRs to form polymorphic contacts with the partners.[177] Such heterogeneity in the bound form is referred to as fuzziness.[175,178] Fuzziness and dynamic binding are universal in the molecular recognition of IDPs/IDRs and are beneficial for their function.[8,174,179] The presence of fuzzy interacting regions adjacent to the main binding elements can regulate binding affinity, specificity, and selectivity.[180–183] Fuzzy regions also facilitate IDPs/IDRs-mediated allosteric communication.[184] Furthermore, transient binding interactions can promote formation of non-native interactions stabilizing the encounter complexes, thus enhance the binding kinetics.[185] Dynamic interactions can also modulate the folding-binding mechanism of IDPs/IDRs.[186] As discussed above, folding of c-Myb is templated by KIX, where transient non-native hydrophobic interactions between c-Myb and KIX populate when the hydrophobic surface in the binding site of KIX is enlarged.[138]

Multivalent dynamic interactions are the main driving forces of LLPS.[187,188] Though many IDPs/IDRs involved in LLPS apply multisite electrostatic and aromatic interactions, dynamic coupled folding-binding interactions mediated by specific recognition elements can also drive LLPS. For example, interactions between SH3 domain and proline-rich motif are involved in the LLPS of the nephrin–NCK–N-WASP system and the RIM–RIM-BP system.[189,190] PDZ domain-mediated binding is required for phase separation of PSD scaffold proteins.[191,192] Multivalent arginine-rich linear motifs interact with the NPM1 pentamer, leading to LLPS.[193] Within the protein-rich droplets, non-native transient interactions are expected to become more populated than in dilute solution. Nevertheless, the specific binding between the recognition motif and the target domain should remain unchanged.

9 | POSTTRANSLATIONAL MODIFICATIONS

IDPs/IDRs are enriched in posttranslational modification (PTM) sites, such as phosphorylation, acetylation, and methylation.[16,194,195] PTMs can regulate molecular recognition of IDPs/IDRs in various ways.[18] For example, PTMs can alter the free energy landscapes of IDPs/IDRs, leading to changes in the conformation ensembles. Bah et al. showed that phosphorylation of 4E-BP2 at T37 and T46 induces folding of 4E-BP2 into a four β-strand structure, sequestering the eIF4E-binding motif and blocking its accessibility to eIF4E.[196] However, the structural changes induced by PTMs could also be subtle, as observed in the N-terminal transactivation domain of p53.[197] PTMs located at the binding interface can directly regulate the interactions between IDPs/IDRs and the targets, for example, phosphorylation will introduce electrostatic interactions between the phosphate moiety and the binding partner.[198] Interestingly, PTMs alter not only the equilibrium conformation ensemble but also conformational exchange among different conformations.[199] Consequently, the binding mechanisms can also be modulated by PTMs.

10 | ALTERNATIVE SPLICING

Alternative splicing (AS) generates various protein forms from a single gene. Previous studies have revealed that AS sites are often located within IDRs which are enriched in molecular recognition motifs.[200,201] As molecular recognition processes of IDPs/IDRs are mainly mediated by short recognition elements, removal of recognition elements by AS will eliminate existed molecular interactions or enable new interactions when competitive interactions are removed. Some proteins contain auto-inhibition segments that mask the binding sites or compete with other molecules for binding.[202] Removal of the auto-inhibition segments by AS will switch the proteins into active states or increase the binding affinities for other molecules. Consequently, removing disordered segments containing different functional or signaling elements allows for rewiring the cellular signaling pathways.[19,26,203]

11 | CONCLUSIONS AND FUTURE PERSPECTIVES

IDPs/IDRs are abundant in all species and involved in vital biological processes. Coupled folding upon binding is an important mode of molecular recognition for IDPs/IDRs. IDPs/IDRs can employ various combinations of conformational selection and induced fit mechanisms and the binding process can be templated by the target and encoded by the IDP/IDR as well. The coupled folding-binding process can also be heterogeneous or fuzzy. While great efforts have been devoted to investigating the complex structures and binding kinetics and affinities, our knowledge on the binding mechanism of IDPs/IDRs remains very limited. Application of advanced kinetic techniques and NMR will provide deeper understanding on the features/mechanisms of molecular recognition of IDPs/IDRs in the future, which may enable rational design of IDP/IDR binding mechanisms.

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REFERENCES

1. Bhowmick A, Brookes DH, Yost SR, et al. Finding our way in the dark proteome. J Am Chem Soc. 2016;138:9730–9742.
2. Gibbs EB, Showalter SA. Quantitative biophysical characterization of intrinsically disordered proteins. Biochemistry. 2015;54:1314–1326.
3. Brucale M, Schuler B, Samori B. Single-molecule studies of intrinsically disordered proteins. Chem Rev. 2014;114:3281–3317.
4. Kragelj J, Ozenne V, Blackledge M, Jensen MR. Conformational propensities of intrinsically disordered proteins from NMR chemical shifts. ChemPhysChem. 2013;14:3034–3045.
5. Uversky VN. Natively unfolded proteins: A point where biology waits for physics. Protein Sci. 2002;11:739–756.
6. Habchi J, Tompa P, Longhi S, Uversky VN. Introducing protein intrinsic disorder. Chem Rev. 2014;114:6561–6588.
7. van der Lee R, Buljan M, Lang B, et al. Classification of intrinsically disordered regions and proteins. Chem Rev. 2014;114:6589–6631.
8. Uversky VN. Functional roles of transiently and intrinsically disordered regions within proteins. FEBS J. 2015;282:1182–1189.
9. Pancsa R, Tompa P. Structural disorder in eukaryotes. PLoS One. 2012;7:e34687.
10. Dunker AK, Obradovic Z, Romero P, Garner EC, Brown CJ. Intrinsic protein disorder in complete genomes. Genome Inform Ser Workshop Genome Inform. 2000;11:161–171.
11. Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF, Jones DT. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J Mol Biol. 2004;337:635–645.
12. 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;30:137–149.
13. 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;72:137–151.
14. Wright PE, Dyson HJ. Intrinsically disordered proteins in cellular signalling and regulation. Nat Rev Mol Cell Biol. 2015;16:18–29.
15. Yruela I, Oldfield CJ, Niklas KJ, Dunker AK. Evidence for a strong correlation between transcription factor protein disorder and organismic complexity. Genome Biol Evol. 2017;9:1248–1265.
16. Xie H, Vucetic S, Iakoucheva LM, et al. Functional anthology of intrinsic disorder. 3. Ligands, post-translational modifications, and diseases associated with intrinsically disordered proteins. J Proteome Res. 2007;6:1917–1932.
17. Xie H, Vucetic S, Iakoucheva LM, et al. Functional anthology of intrinsic disorder. 1. Biological processes and functions of proteins with long disordered regions. J Proteome Res. 2007;6:1882–1898.
18. Bah A, Forman-Kay JD. Modulation of intrinsically disordered protein function by post-translational modifications. J Biol Chem. 2016;291:6696–6705.
19. Buljan M, Chalancon G, Dunker AK, et al. Alternative splicing of intrinsically disordered regions and rewiring of protein interactions. Curr Opin Struct Biol. 2013;23:443–450.
20. Zagrovic B, Bartonek L, Polyansky AA. RNA-protein interactions in an unstructured context. FEBS Lett. 2018;592:2901–2916.
21. Staby L, O'Shea C, Willemoes M, Theisen F, Kragelund BB, Skriver K. Eukaryotic transcription factors: Paradigms of protein intrinsic disorder. Biochem J. 2017;474:2509–2532.
22. Dyson HJ, Wright PE. Role of intrinsic protein disorder in the function and interactions of the transcriptional coactivators CREB-binding protein (CBP) and p300. J Biol Chem. 2016;291:6714–6722.
23. Tantos A, Han KH, Tompa P. Intrinsic disorder in cell signaling and gene transcription. Mol Cell Endocrinol. 2012;348:457–465.
24. Moldoveanu T, Follis AV, Kriwacki RW, Green DR. Many players in BCL-2 family affairs. Trends Biochem Sci. 2014;39:101–111.
25. Wang Y, Bugge K, Kragelund BB, Lindorff-Larsen K. Role of protein dynamics in transmembrane receptor signalling. Curr Opin Struct Biol. 2018;48:74–82.
26. Zhou J, Zhao S, Dunker AK. Intrinsically disordered proteins link alternative splicing and post-translational modifications to complex cell signaling and regulation. J Mol Biol. 2018;430:2342–2359.
27. Pancsa R, Tompa P. Coding regions of intrinsic disorder accommodate parallel functions. Trends Biochem Sci. 2016;41:898–906.
28. Varadi M, Zsolyomi F, Guharoy M, Tompa P. Functional advantages of conserved intrinsic disorder in RNA-binding proteins. PLoS One. 2015;10:e0139731.
29. Berlow RB, Dyson HJ, Wright PE. Expanding the paradigm: Intrinsically disordered proteins and allosteric regulation. J Mol Biol. 2018;430:2309–2320.
30. Berlow RB, Dyson HJ, Wright PE. Functional advantages of dynamic protein disorder. FEBS Lett. 2015;589:2433–2440.

31. Jakob U, Kriwacki R, Uversky VN. Conditionally and transiently disordered proteins: Awakening cryptic disorder to regulate protein function. Chem Rev. 2014;114:6779–6805.
32. Midic U, Oldfield CJ, Dunker AK, Obradovic Z, Uversky VN. Protein disorder in the human diseasome: Unfoldomics of human genetic diseases. BMC Genomics. 2009;10:S12.
33. Vavouri T, Semple JI, Garcia-Verdugo R, Lehner B. Intrinsic protein disorder and interaction promiscuity are widely associated with dosage sensitivity. Cell. 2009;138:198–208.
34. Uyar B, Weatheritt RJ, Dinkel H, Davey NE, Gibson TJ. Proteome-wide analysis of human disease mutations in short linear motifs: Neglected players in cancer? Mol Biosyst. 2014;10:2626–2642.
35. Meyer K, Kirchner M, Uyar B, et al. Mutations in disordered regions can cause disease by creating dileucine motifs. Cell. 2018;175:239–253.
36. Babu MM. The contribution of intrinsically disordered regions to protein function, cellular complexity, and human disease. Biochem Soc Trans. 2016;44:1185–1200.
37. Iakoucheva LM, Brown CJ, Lawson JD, Obradović Z, Dunker AK. Intrinsic disorder in cell-signaling and cancer-associated proteins. J Mol Biol. 2002;323:573–584.
38. Uversky VN, Oldfield CJ, Dunker AK. Intrinsically disordered proteins in human diseases: Introducing the D2 concept. Annu Rev Biophys. 2008;37:215–246.
39. Uversky VN, Dave V, Iakoucheva LM, et al. Pathological unfoldomics of uncontrolled chaos: Intrinsically disordered proteins and human diseases. Chem Rev. 2014;114:6844–6879.
40. Kulkarni P, Uversky VN. Intrinsically disordered proteins in chronic diseases. Biomolecules. 2019;9:147.
41. Uversky VN. Intrinsic disorder, protein-protein interactions, and disease. Adv Protein Chem Struct Biol. 2018;110:85–121.
42. Tuu-Szabo B, Hoffka G, Duro N, Fuxreiter M. Altered dynamics may drift pathological fibrillization in membraneless organelles. Biochim Biophys Acta Proteins Proteom. 2019;1867:988–998.
43. Elbaum-Garfinkle S. Matter over mind: Liquid phase separation and neurodegeneration. J Biol Chem. 2019;294:7160–7168.
44. Oates ME, Romero P, Ishida T, et al. D^2P^2: Database of disordered protein predictions. Nucleic Acids Res. 2013;41:D508–D516.
45. Boeynaems S, Alberti S, Fawzi NL, et al. Protein phase separation: A new phase in cell biology. Trends Cell Biol. 2018;28:420–435.
46. Li XH, Chavali PL, Pancsa R, Chavali S, Babu MM. Function and regulation of phase-separated biological condensates. Biochemistry. 2018;57:2452–2461.
47. Shin Y, Brangwynne CP. Liquid phase condensation in cell physiology and disease. Science. 2017;357:eaaf4382.
48. Aguzzi A, Altmeyer M. Phase separation: Linking cellular compartmentalization to disease. Trends Cell Biol. 2016;26:547–558.
49. Uversky VN, Kuznetsova IM, Turoverov KK, Zaslavsky B. Intrinsically disordered proteins as crucial constituents of cellular aqueous two phase systems and coacervates. FEBS Lett. 2015;589:15–22.
50. Darling AL, Liu Y, Oldfield CJ, Uversky VN. Intrinsically disordered proteome of human membrane-less organelles. Proteomics. 2018;18:e1700193.
51. Uversky VN. Intrinsically disordered proteins in overcrowded milieu: Membrane-less organelles, phase separation, and intrinsic disorder. Curr Opin Struct Biol. 2017;44:18–30.
52. 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.
53. Turoverov KK, Kuznetsova IM, Fonin AV, Darling AL, Zaslavsky BY, Uversky VN. Stochasticity of biological soft matter: Emerging concepts in intrinsically disordered proteins and biological phase separation. Trends Biochem Sci. 2019;44:716–728.
54. Minde DP, Dunker AK, Lilley KS. Time, space, and disorder in the expanding proteome universe. Proteomics. 2017;17:1600399.
55. Burger VM, Nolasco DO, Stultz CM. Expanding the range of protein function at the far end of the order-structure continuum. J Biol Chem. 2016;291:6706–6713.
56. Tompa P, Schad E, Tantos A, Kalmar L. Intrinsically disordered proteins: Emerging interaction specialists. Curr Opin Struct Biol. 2015;35:49–59.
57. Uversky VN. p53 proteoforms and intrinsic disorder: An illustration of the protein structure-function continuum concept. Int J Mol Sci. 2016;17:1874.
58. Liu ZR, Huang YQ. Advantages of proteins being disordered. Protein Sci. 2014;23:539–550.
59. Mohan A, Oldfield CJ, Radivojac P, et al. Analysis of molecular recognition features (MoRFs). J Mol Biol. 2006;362:1043–1059.
60. Davey NE, Van Roey K, Weatheritt RJ, et al. Attributes of short linear motifs. Mol Biosyst. 2012;8:268–281.
61. Seo MH, Kim PM. The present and the future of motif-mediated protein-protein interactions. Curr Opin Struct Biol. 2018;50:162–170.
62. Kim DH, Han KH. Transient secondary structures as general target-binding motifs in intrinsically disordered proteins. Int J Mol Sci. 2018;19:3614.
63. Oldfield CJ, Cheng YG, Cortese MS, Romero P, Uversky VN, Dunker AK. Coupled folding and binding with a-helix-forming molecular recognition elements. Biochemistry. 2005;44:12454–12470.
64. Meng F, Uversky VN, Kurgan L. Comprehensive review of methods for prediction of intrinsic disorder and its molecular functions. Cell Mol Life Sci. 2017;74:3069–3090.
65. Mészáros B, Simon I, Dosztányi Z. Prediction of protein binding regions in disordered proteins. PLoS Comput Biol. 2009;5:e1000376.
66. Dosztanyi Z, Meszaros B, Simon I. ANCHOR: Web server for predicting protein binding regions in disordered proteins. Bioinformatics. 2009;25:2745–2746.
67. Meszaros B, Dosztanyi Z, Simon I. Disordered binding regions and linear motifs--bridging the gap between two models of molecular recognition. PLoS One. 2012;7:e46829.
68. Meszaros B, Erdos G, Dosztanyi Z. IUPred2A: Context-dependent prediction of protein disorder as a function of redox state and protein binding. Nucleic Acids Res. 2018;46:W329–W337.
69. Wright PE, Dyson HJ. Linking folding and binding. Curr Opin Struct Biol. 2009;19:31–38.
70. Dyson HJ, Wright PE. Coupling of folding and binding for unstructured proteins. Curr Opin Struct Biol. 2002;12:54–60.
71. Milles S, Salvi N, Blackledge M, Jensen MR. 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.

72. LeBlanc SJ, Kulkarni P, Weninger KR. Single molecule FRET: A powerful tool to study intrinsically disordered proteins. Biomolecules. 2018;8:140.
73. Schneider R, Blackledge M, Jensen MR. Elucidating binding mechanisms and dynamics of intrinsically disordered protein complexes using NMR spectroscopy. Curr Opin Struct Biol. 2018;54:10–18.
74. Stuchfield D, Barran P. Unique insights to intrinsically disordered proteins provided by ion mobility mass spectrometry. Curr Opin Chem Biol. 2018;42:177–185.
75. Shammas SL, Crabtree MD, Dahal L, Wicky BI, Clarke J. Insights into coupled folding and binding mechanisms from kinetic studies. J Biol Chem. 2016;291:6689–6695.
76. Ciemny MP, Badaczewska-Dawid AE, Pikuzinska M, Kolinski A, Kmiecik S. Modeling of disordered protein structures using Monte Carlo simulations and knowledge-based statistical force fields. Int J Mol Sci. 2019;20:606.
77. Das P, Matysiak S, Mittal J. Looking at the disordered proteins through the computational microscope. ACS Cent Sci. 2018;4:534–542.
78. Best RB. Computational and theoretical advances in studies of intrinsically disordered proteins. Curr Opin Struct Biol. 2017;42:147–154.
79. Levine ZA, Shea JE. Simulations of disordered proteins and systems with conformational heterogeneity. Curr Opin Struct Biol. 2017;43:95–103.
80. Chong SH, Chatterjee P, Ham S. Computer simulations of intrinsically disordered proteins. Annu Rev Phys Chem. 2017;68:117–134.
81. Chen T, Song J, Chan HS. Theoretical perspectives on nonnative interactions and intrinsic disorder in protein folding and binding. Curr Opin Struct Biol. 2015;30:32–42.
82. Baker CM, Best RB. Insights into the binding of intrinsically disordered proteins from molecular dynamics simulation. WIREs Comput Mol Sci. 2014;4:182–198.
83. Shoemaker BA, Portman JJ, Wolynes PG. Speeding molecular recognition by using the folding funnel: The fly-casting mechanism. Proc Natl Acad Sci U S A. 2000;97:8868–8873.
84. Trizac E, Levy Y, Wolynes PG. Capillarity theory for the fly-casting mechanism. Proc Natl Acad Sci U S A. 2010;107:2746–2750.
85. Huang Y, Liu Z. Kinetic advantage of intrinsically disordered proteins in coupled folding-binding process: A critical assessment of the "fly-casting" mechanism. J Mol Biol. 2009;393:1143–1159.
86. Iesmantavicius V, Dogan J, Jemth P, Teilum K, Kjaergaard M. Helical propensity in an intrinsically disordered protein accelerates ligand binding. Angew Chem Int Ed Engl. 2014;53:1548–1551.
87. Rogers JM, Wong CT, Clarke J. Coupled folding and binding of the disordered protein PUMA does not require particular residual structure. J Am Chem Soc. 2014;136:5197–5200.
88. Arai M, Sugase K, Dyson HJ, Wright PE. Conformational propensities of intrinsically disordered proteins influence the mechanism of binding and folding. Proc Natl Acad Sci U S A. 2015;112:9614–9619.
89. Schmidtgall B, Chaloin O, Bauer V, Sumyk M, Birck C, Torbeev V. Dissecting mechanism of coupled folding and binding of an intrinsically disordered protein by chemical synthesis of conformationally constrained analogues. Chem Commun. 2017;53:7369–7372.
90. Liu X, Chen J, Chen J. Residual structure accelerates binding of intrinsically disordered ACTR by promoting efficient folding upon encounter. J Mol Biol. 2019;431:422–432.
91. Umezawa K, Ohnuki J, Higo J, Takano M. Intrinsic disorder accelerates dissociation rather than association. Proteins. 2016;84:1124–1133.
92. Narasumani M, Harrison PM. Bioinformatical parsing of folding-on-binding proteins reveals their compositional and evolutionary sequence design. Sci Rep. 2015;5:18586.
93. Dogan J, Jonasson J, Andersson E, Jemth P. Binding rate constants reveal distinct features of disordered protein domains. Biochemistry. 2015;54:4741–4750.
94. Chemes LB, Sanchez IE, de Prat-Gay G. Kinetic recognition of the retinoblastoma tumor suppressor by a specific protein target. J Mol Biol. 2011;412:267–284.
95. Shammas SL, Travis AJ, Clarke J. Allostery within a transcription coactivator is predominantly mediated through dissociation rate constants. Proc Natl Acad Sci U S A. 2014;111:12055–12060.
96. Lindstrom I, Dogan J. Native hydrophobic binding interactions at the transition state for association between the TAZ1 domain of CBP and the disordered TAD-STAT2 are not a requirement. Biochemistry. 2017;56:4145–4153.
97. Ganguly D, Otieno S, Waddell B, Iconaru L, Kriwacki RW, Chen J. Electrostatically accelerated coupled binding and folding of intrinsically disordered proteins. J Mol Biol. 2012;422:674–684.
98. Papadakos G, Sharma A, Lancaster LE, et al. Consequences of inducing intrinsic disorder in a high-affinity protein-protein interaction. J Am Chem Soc. 2015;137:5252–5255.
99. Rogers JM, Steward A, Clarke J. Folding and binding of an intrinsically disordered protein: Fast, but not "diffusion-limited". J Am Chem Soc. 2013;135:1415–1422.
100. Ou L, Matthews M, Pang X, Zhou HX. The dock-and-coalesce mechanism for the association of a WASP disordered region with the Cdc42 GTPase. FEBS J. 2017;284:3381–3391.
101. Aberg E, Karlsson OA, Andersson E, Jemth P. Binding kinetics of the intrinsically disordered p53 family transactivation domains and MDM2. J Phys Chem B. 2018;122:6899–6905.
102. Shammas SL, Travis AJ, Clarke J. Remarkably fast coupled folding and binding of the intrinsically disordered transactivation domain of cMyb to CBP KIX. J Phys Chem B. 2013;117:13346–13356.
103. Gallagher E, Song JM, Menon A, Mishra L, Chmiel AF, Garner AL. Consideration of binding kinetics in the design of stapled peptide mimics of the disordered proteins eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and eukaryotic translation initiation factor 4G (eIF4G). J Med Chem. 2019;62:4967–4978.
104. Chu X, Wang Y, Gan L, et al. Importance of electrostatic interactions in the association of intrinsically disordered histone chaperone Chz1 and histone H2A.Z-H2B. PLoS Comput Biol. 2012;8:e1002608.
105. Ganguly D, Zhang W, Chen J. Electrostatically accelerated encounter and folding for facile recognition of intrinsically disordered proteins. PLoS Comput Biol. 2013;9:e1003363.
106. Pang X, Zhou HX. Mechanism and rate constants of the Cdc42 GTPase binding with intrinsically disordered effectors. Proteins. 2016;84:674–685.

107. Tsai MY, Zheng W, Balamurugan D, et al. Electrostatics, structure prediction, and the energy landscapes for protein folding and binding. Protein Sci. 2016;25:255–269.
108. Chu WT, Clarke J, Shammas SL, Wang J. Role of non-native electrostatic interactions in the coupled folding and binding of PUMA with mcl-1. PLoS Comput Biol. 2017;13:e1005468.
109. Sturzenegger F, Zosel F, Holmstrom ED, et al. Transition path times of coupled folding and binding reveal the formation of an encounter complex. Nat Commun. 2018;9:4708.
110. Kim JY, Meng F, Yoo J, Chung HS. Diffusion-limited association of disordered protein by non-native electrostatic interactions. Nat Commun. 2018;9:4707.
111. Dunker AK, Oldfield CJ. Back to the future: Nuclear magnetic resonance and bioinformatics studies on intrinsically disordered proteins. Adv Exp Med Biol. 2015;870:1–34.
112. Mollica L, Bessa LM, Hanoulle X, Jensen MR, Blackledge M, Schneider R. Binding mechanisms of intrinsically disordered proteins: Theory, simulation, and experiment. Front Mol Biosci. 2016;3:52.
113. Dogan J, Gianni S, Jemth P. The binding mechanisms of intrinsically disordered proteins. Phys Chem Chem Phys. 2014;16:6323–6331.
114. Weikl TR, von Deuster C. Selected-fit versus induced-fit protein binding: Kinetic differences and mutational analysis. Proteins. 2009;75:104–110.
115. Vogt AD, Di Cera E. Conformational selection or induced fit? A critical appraisal of the kinetic mechanism. Biochemistry. 2012;51:5894–5902.
116. Gianni S, Dogan J, Jemth P. Distinguishing induced fit from conformational selection. Biophys Chem. 2014;189:33–39.
117. Paul F, Weikl TR. How to distinguish conformational selection and induced fit based on chemical relaxation rates. PLoS Comput Biol. 2016;12:e1005067.
118. Chakraborty P, Di Cera E. Induced fit is a special case of conformational selection. Biochemistry. 2017;56:2853–2859.
119. Hammes GG, Chang YC, Oas TG. Conformational selection or induced fit: A flux description of reaction mechanism. Proc Natl Acad Sci U S A. 2009;106:13737–13741.
120. Greives N, Zhou HX. Both protein dynamics and ligand concentration can shift the binding mechanism between conformational selection and induced fit. Proc Natl Acad Sci U S A. 2014;111:10197–10202.
121. Daniels KG, Tonthat NK, McClure DR, et al. Ligand concentration regulates the pathways of coupled protein folding and binding. J Am Chem Soc. 2014;136:822–825.
122. Zhou HX. From induced fit to conformational selection: A continuum of binding mechanism controlled by the timescale of conformational transitions. Biophys J. 2010;98:L15–L17.
123. Liu C, Wang T, Bai Y, Wang J. Electrostatic forces govern the binding mechanism of intrinsically disordered histone chaperones. PLoS One. 2017;12:e0178405.
124. Daniels KG, Suo Y, Oas TG. Conformational kinetics reveals affinities of protein conformational states. Proc Natl Acad Sci U S A. 2015;112:9352–9357.
125. Weikl TR, Paul F. Conformational selection in protein binding and function. Protein Sci. 2014;23:1508–1518.
126. Choi UB, Sanabria H, Smirnova T, Bowen ME, Weninger KR. Spontaneous switching among conformational ensembles in intrinsically disordered proteins. Biomolecules. 2019;9:114.
127. Salvi N, Abyzov A, Blackledge M. Multi-timescale dynamics in intrinsically disordered proteins from NMR relaxation and molecular simulation. J Phys Chem Lett. 2016;7:2483–2489.
128. Jain N, Narang D, Bhasne K, et al. Direct observation of the intrinsic backbone torsional mobility of disordered proteins. Biophys J. 2016;111:768–774.
129. Soranno A, Longhi R, Bellini T, Buscaglia M. Kinetics of contact formation and end-to-end distance distributions of swollen disordered peptides. Biophys J. 2009;96:1515–1528.
130. Rogers JM, Oleinikovas V, Shammas SL, et al. Interplay between partner and ligand facilitates the folding and binding of an intrinsically disordered protein. Proc Natl Acad Sci U S A. 2014;111:15420–15425.
131. Gianni S, Morrone A, Giri R, Brunori M. A folding-after-binding mechanism describes the recognition between the transactivation domain of c-Myb and the KIX domain of the CREB-binding protein. Biochem Biophys Res Commun. 2012;428:205–209.
132. Gianni S, Dogan J, Jemth P. Coupled binding and folding of intrinsically disordered proteins: What can we learn from kinetics? Curr Opin Struct Biol. 2016;36:18–24.
133. Dogan J, Schmidt T, Mu X, Engstrom A, Jemth P. Fast association and slow transitions in the interaction between two intrinsically disordered protein domains. J Biol Chem. 2012;287:34316–34324.
134. Dosnon M, Bonetti D, Morrone A, et al. Demonstration of a folding after binding mechanism in the recognition between the measles virus NTAIL and X domains. ACS Chem Biol. 2015;10:795–802.
135. Espinoza-Fonseca LM. Reconciling binding mechanisms of intrinsically disordered proteins. Biochem Biophys Res Commun. 2009;382:479–482.
136. Schneider R, Maurin D, Communie G, et al. Visualizing the molecular recognition trajectory of an intrinsically disordered protein using multinuclear relaxation dispersion NMR. J Am Chem Soc. 2015;137:1220–1229.
137. Lindstrom I, Andersson E, Dogan J. The transition state structure for binding between TAZ1 of CBP and the disordered Hif-1a CAD. Sci Rep. 2018;8:7872.
138. Toto A, Camilloni C, Giri R, Brunori M, Vendruscolo M, Gianni S. Molecular recognition by templated folding of an intrinsically disordered protein. Sci Rep. 2016;6:21994.
139. Giri R, Morrone A, Toto A, Brunori M, Gianni S. Structure of the transition state for the binding of c-Myb and KIX highlights an unexpected order for a disordered system. Proc Natl Acad Sci U S A. 2013;110:14942–14947.
140. Dahal L, Kwan TOC, Shammas SL, Clarke J. pKID binds to KIX via an unstructured transition state with nonnative interactions. Biophys J. 2017;113:2713–2722.
141. Crabtree MD, Mendonca C, Bubb QR, Clarke J. Folding and binding pathways of BH3-only proteins are encoded within their intrinsically disordered sequence, not templated by partner proteins. J Biol Chem. 2018;293:9718–9723.
142. Toto A, Gianni S. Mutational analysis of the binding-induced folding reaction of the mixed-lineage leukemia protein to the KIX domain. Biochemistry. 2016;55:3957–3962.
143. Haq SR, Chi CN, Bach A, et al. Side-chain interactions form late and cooperatively in the binding reaction between disordered peptides and PDZ domains. J Am Chem Soc. 2012;134:599–605.
144. Bachmann A, Wildemann D, Praetorius F, Fischer G, Kiefhaber T. Mapping backbone and side-chain interactions in

the transition state of a coupled protein folding and binding reaction. Proc Natl Acad Sci U S A. 2011;108:3952–3957.
145. Bonetti D, Troilo F, Toto A, Brunori M, Longhi S, Gianni S. Analyzing the folding and binding steps of an intrinsically disordered protein by protein engineering. Biochemistry. 2017;56:3780–3786.
146. Hill SA, Kwa LG, Shammas SL, Lee JC, Clarke J. Mechanism of assembly of the non-covalent spectrin tetramerization domain from intrinsically disordered partners. J Mol Biol. 2014;426:21–35.
147. Karlsson E, Andersson E, Dogan J, Gianni S, Jemth P, Camilloni C. A structurally heterogeneous transition state underlies coupled binding and folding of disordered proteins. J Biol Chem. 2019;294:1230–1239.
148. Dogan J, Mu X, Engstrom A, Jemth P. The transition state structure for coupled binding and folding of disordered protein domains. Sci Rep. 2013;3:2076.
149. Karlsson OA, Chi CN, Engstrom A, Jemth P. The transition state of coupled folding and binding for a flexible beta-finger. J Mol Biol. 2012;417:253–261.
150. Zhou HX, Pang X, Lu C. Rate constants and mechanisms of intrinsically disordered proteins binding to structured targets. Phys Chem Chem Phys. 2012;14:10466–10476.
151. Wu D, Zhou HX. Designed mutations alter the binding pathways of an intrinsically disordered protein. Sci Rep. 2019;9:6172.
152. Staneva I, Huang Y, Liu Z, Wallin S. Binding of two intrinsically disordered peptides to a multi-specific protein: A combined Monte Carlo and molecular dynamics study. PLoS Comput Biol. 2012;8:e1002682.
153. Bonetti D, Troilo F, Brunori M, Longhi S, Gianni S. How robust is the mechanism of folding-upon-binding for an intrinsically disordered protein? Biophys J. 2018;114:1889–1894.
154. Ellis RJ, Minton AP. Cell biology: Join the crowd. Nature. 2003;425:27–28.
155. Zhou HX, Rivas G, Minton AP. Macromolecular crowding and confinement: Biochemical, biophysical, and potential physiological consequences. Annu Rev Biophys. 2008;37:375–397.
156. Fonin AV, Darling AL, Kuznetsova IM, Turoverov KK, Uversky VN. Intrinsically disordered proteins in crowded milieu: When chaos prevails within the cellular gumbo. Cell Mol Life Sci. 2018;75:3907–3929.
157. Kuznetsova IM, Turoverov KK, Uversky VN. What macromolecular crowding can do to a protein. Int J Mol Sci. 2014;15:23090–23140.
158. Soranno A, Koenig I, Borgia MB, et al. Single-molecule spectroscopy reveals polymer effects of disordered proteins in crowded environments. Proc Natl Acad Sci U S A. 2014;111:4874–4879.
159. Kang H, Pincus PA, Hyeon C, Thirumalai D. Effects of macromolecular crowding on the collapse of biopolymers. Phys Rev Lett. 2015;114:068303.
160. Miller CM, Kim YC, Mittal J. Protein composition determines the effect of crowding on the properties of disordered proteins. Biophys J. 2016;111:28–37.
161. Bai J, Liu M, Pielak GJ, Li C. Macromolecular and small molecular crowding have similar effects on alpha-synuclein structure. Chem Phys Chem. 2017;18:55–58.
162. Balu R, Mata JP, Knott R, et al. Effects of crowding and environment on the evolution of conformational ensembles of the multi-stimuli-responsive intrinsically disordered protein, Rec1-resilin: A small-angle scattering investigation. J Phys Chem B. 2016;120:6490–6503.
163. Goldenberg DP, Argyle B. Minimal effects of macromolecular crowding on an intrinsically disordered protein: A small-angle neutron scattering study. Biophys J. 2014;106:905–914.
164. Szasz CS, Alexa A, Toth K, Rakacs M, Langowski J, Tompa P. Protein disorder prevails under crowded conditions. Biochemistry. 2011;50:5834–5844.
165. Dedmon MM, Patel CN, Young GB, Pielak GJ. FlgM gains structure in living cells. Proc Natl Acad Sci U S A. 2002;99:12681–12684.
166. Cook EC, Creamer TP. Calcineurin in a crowded world. Biochemistry. 2016;55:3092–3101.
167. Rusinga FI, Weis DD. Soft interactions and volume exclusion by polymeric crowders can stabilize or destabilize transient structure in disordered proteins depending on polymer concentration. Proteins. 2017;85:1468–1479.
168. Flaugh SL, Lumb KJ. Effects of macromolecular crowding on the intrinsically disordered proteins c-Fos and $p27^{Kip1}$. Biomacromolecules. 2001;2:538–540.
169. Wang Y, Benton LA, Singh V, Pielak GJ. Disordered protein diffusion under crowded conditions. J Phys Chem Lett. 2012;3:2703–2706.
170. Li CG, Charlton LM, Lakkavaram A, et al. Differential dynamical effects of macromolecular crowding on an intrinsically disordered protein and a globular protein: Implications for in-cell NMR spectroscopy. J Am Chem Soc. 2008;130:6310–6311.
171. Banks A, Qin S, Weiss KL, Stanley CB, Zhou HX. Intrinsically disordered protein exhibits both compaction and expansion under macromolecular crowding. Biophys J. 2018;114:1067–1079.
172. Hoffman L, Wang X, Sanabria H, Cheung MS, Putkey JA, Waxham MN. Relative cosolute size influences the kinetics of protein-protein interactions. Biophys J. 2015;109:510–520.
173. Kim YC, Bhattacharya A, Mittal J. Macromolecular crowding effects on coupled folding and binding. J Phys Chem B. 2014;118:12621–12629.
174. Olsen JG, Teilum K, Kragelund BB. Behaviour of intrinsically disordered proteins in protein-protein complexes with an emphasis on fuzziness. Cell Mol Life Sci. 2017;74:3175–3183.
175. Fuxreiter M. Fuzziness in protein interactions-a historical perspective. J Mol Biol. 2018;430:2278–2287.
176. Lindstrom I, Dogan J. Dynamics, conformational entropy, and frustration in protein-protein interactions involving an intrinsically disordered protein domain. ACS Chem Biol. 2018;13:1218–1227.
177. Borgia A, Borgia MB, Bugge K, et al. Extreme disorder in an ultrahigh-affinity protein complex. Nature. 2018;555:61–66.
178. Tompa P, Fuxreiter M. Fuzzy complexes: Polymorphism and structural disorder in protein-protein interactions. Trends Biochem Sci. 2008;33:2–8.
179. Sharma R, Raduly Z, Miskei M, Fuxreiter M. Fuzzy complexes: Specific binding without complete folding. FEBS Lett. 2015;589:2533–2542.
180. Graham TA, Ferkey DM, Mao F, Kimelman D, Xu W. Tcf4 can specifically recognize beta-catenin using alternative conformations. Nat Struct Biol. 2001;8:1048–1052.
181. Smet-Nocca C, Wieruszeski JM, Chaar V, Leroy A, Benecke A. The thymine-DNA glycosylase regulatory domain: Residual structure and DNA binding. Biochemistry. 2008;47:6519–6530.

182. Liu Y, Matthews KS, Bondos SE. Internal regulatory interactions determine DNA binding specificity by a Hox transcription factor. J Mol Biol. 2009;390:760–774.
183. Wang Y, Fisher JC, Mathew R, et al. Intrinsic disorder mediates the diverse regulatory functions of the Cdk inhibitor p21. Nat Chem Biol. 2011;7:214–221.
184. Miskei M, Gregus A, Sharma R, Duro N, Zsolyomi F, Fuxreiter M. Fuzziness enables context dependence of protein interactions. FEBS Lett. 2017;591:2682–2695.
185. Toth-Petroczy A, Simon I, Fuxreiter M, Levy Y. Disordered tails of homeodomains facilitate DNA recognition by providing a trade-off between folding and specific binding. J Am Chem Soc. 2009;131:15084–15085.
186. Fuxreiter M. Fold or not to fold upon binding - does it really matter? Curr Opin Struct Biol. 2019;54:19–25.
187. Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular condensates: Organizers of cellular biochemistry. Nat Rev Mol Cell Biol. 2017;18:285–298.
188. Wu H, Fuxreiter M. The structure and dynamics of higher-order assemblies: Amyloids, signalosomes, and granules. Cell. 2016; 165:1055–1066.
189. Li P, Banjade S, Cheng HC, et al. Phase transitions in the assembly of multivalent signalling proteins. Nature. 2012;483:336–340.
190. Wu X, Cai Q, Shen Z, et al. RIM and RIM-BP form presynaptic active-zone-like condensates via phase separation. Mol Cell. 2019;73:971–984.
191. Zeng M, Shang Y, Araki Y, Guo T, Huganir RL, Zhang M. Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity. Cell. 2016;166:1163–1175.
192. Zeng M, Chen X, Guan D, et al. Reconstituted postsynaptic density as a molecular platform for understanding synapse formation and plasticity. Cell. 2018;174:1172–1187.
193. Mitrea DM, Cika JA, Guy CS, et al. Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA. Elife. 2016;5: e13571.
194. Darling AL, Uversky VN. Intrinsic disorder and posttranslational modifications: The darker side of the biological dark matter. Front Genet. 2018;9:158.
195. Pejaver V, Hsu WL, Xin F, Dunker AK, Uversky VN, Radivojac P. The structural and functional signatures of proteins that undergo multiple events of post-translational modification. Protein Sci. 2014;23:1077–1093.
196. Bah A, Vernon RM, Siddiqui Z, et al. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. Nature. 2015;519:106–109.
197. Levy R, Gregory E, Borcherds W, Daughdrill G. p53 phosphomimetics preserve transient secondary structure but reduce binding to Mdm2 and MdmX. Biomolecules. 2019;9:83.
198. Joo Y, Schumacher B, Landrieu I, et al. Involvement of 14-3-3 in tubulin instability and impaired axon development is mediated by tau. FASEB J. 2015;29:4133–4144.
199. Zhao L, Ouyang Y, Li Q, Zhang Z. Modulation of p53 N-terminal transactivation domain 2 conformation ensemble and kinetics by phosphorylation. J Biomol Struct Dyn. 2019;1–11. https://doi.org/10.1080/07391102.2019.1637784
200. Romero PR, Zaidi S, Fang YY, et al. Alternative splicing in concert with protein intrinsic disorder enables increased functional diversity in multicellular organisms. Proc Natl Acad Sci U S A. 2006;103:8390–8395.
201. Weatheritt RJ, Davey NE, Gibson TJ. Linear motifs confer functional diversity onto splice variants. Nucleic Acids Res. 2012;40:7123–7131.
202. Trudeau T, Nassar R, Cumberworth A, Wong ET, Woollard G, Gsponer J. Structure and intrinsic disorder in protein autoinhibition. Structure. 2013;21:332–341.
203. Buljan M, Chalancon G, Eustermann S, et al. Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. Mol Cell. 2012;46:871–883.

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