Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Structural Biology

Unveiling induced folding of intrinsically disordered proteins – Protein engineering, frustration and emerging themes

Francesca Malagrinò, Awa Diop, Livia Pagano, Caterina Nardella, Angelo Toto and Stefano Gianni

Abstract

Intrinsically disordered proteins (IDPs) can be generally described as a class of proteins that lack a well-defined ordered structure in isolation at physiological conditions. Upon binding to their physiological ligands, IDPs typically undergo a disorder-to-order transition, which may or may not lead to the complete folding of the IDP. In this short review, we focus on some of the key findings pertaining to the mechanisms of such induced folding. In particular, first we describe the general features of the reaction; then, we discuss some of the most remarkable findings obtained from applying protein engineering in synergy with kinetic studies to induced folding; and finally, we offer a critical view on some of the emerging themes when considering the structural heterogeneity of IDPs vis-à-vis to their inherent frustration.

Addresses

Istituto Pasteur, Fondazione Cenci Bolognetti, Dipartimento di Scienze Biochimiche "A. Rossi Fanelli" and Istituto di Biologia e Patologia Molecolari Del CNR, Sapienza Università, di Roma, 00185, Rome, Italy

Corresponding authors: Gianni, Stefano (stefano.gianni@uniroma1.it); Toto, Angelo (angelo.toto@uniroma1.it)

Current Opinion in Structural Biology 2022, **72**:153–160

This review comes from a themed issue on **Folding and Binding**

Edited by **Fabrizio Chiti** and **Anna Sablina**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.sbi.2021.11.004

0959-440X/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

"Chemistry is neither chess nor geometry, whatever x-ray physics may be." With this vitriolic statement, a commentary entitled "Poor Common Salt!", published in Nature in 1927 [1], conveyed the criticism of x-ray diffraction experiments by Sir. Lawrence Bragg on NaCl [2]. Such initial skepticism was also present when x-ray crystallography was extended to biological macromolecules, highlighting concerns about both the inherent difficulty of the method and possible artefacts induced by trapping proteins in an artificial crystalline state.

Since its infancy, however, the rapid success of protein crystallography overcame such technical doubts and the invaluable information provided by this technique soon established its pivotal role in science. Nevertheless, from the moment in which the function of haemoglobin could be explained by comparing its R and T states [3], it immediately became clear how proteins could be fully understood only when their dynamic properties were also taken into account. Indeed, research during the following decades corroborated that the dynamics of proteins are critical to understand their function.

Five decades after the first determination of an X-ray protein structure [4], the discovery that up to 30% of the human proteome is disordered in its functional state has completely revolutionized the structure–function dogma [5]. This finding originally led to the view that disordered proteins were a sort of specific class of molecules "breaking the protein rules" [6] or displaying "unusual biophysics" [7], calling therefore for a rigorous description of their behaviour as well as in solving the quest of the importance and value of disorder in the protein world.

The collaborative efforts of experimentalists and theoreticians have recently tremendously contributed to our understanding of the structural and functional properties of intrinsically disordered proteins (IDPs). In fact, given their abundance and importance in several critical cellular processes, much effort has been devoted in the rigorous study of this type of proteins [5,8–18]. In this review, we attempt to offer a critical view on some of these key findings, posing particular attention on the information accumulated on the mechanisms of binding and recognition of IDPs, as well as on their key differences and similarities as compared to globular proteins.

Folding upon binding of IDPs

To a first approximation, an IDP may be defined as a protein, or a protein segment, which lacks a well-defined ordered structure in isolation at physiological conditions [19]. In this context, it is worth noticing how the

complicated nature of the cellular environment poses the definition of 'physiological conditions' as a complex matter. In fact, whilst the cell is a crowded medium comprising osmolytes, carbohydrates, nucleic acids and proteins, there is sometimes the shallow tendency to assume that a buffer solution at physiological pH and ionic strength resembles the 'physiological conditions'. This issue has been often taken as an opportunity to criticize the existence per se of IDPs, whose apparent disorder has been suggested to arise from an experimental artefact due to the in vitro conditions, which do not sufficiently mimic the real cellular conditions. Despite these skepticisms, a wealth of experimental data accumulated over the past two decades pinpoints how IDPs maintain their disordered state within the crowded cellular environment [20], reinforcing the importance to study and understand this class of proteins as well as in highlighting the potential values of being disordered.

When recognizing and binding a physiological partner, an IDP may encounter conformational transitions [19]. In some cases, these structural changes might be so pronounced that the IDP effectively undergoes a disorder-to-order transition and folds upon binding to its substrate. However, not all IDPs are capable of folding, and in other cases, the resulting complex maintains a considerable level of disorder. Notably, even the latter cases, despite retaining disorder, might correspond to very tight complexes, displaying nM affinities [21] and indicating that the level of disorder found in a complex is not related to the apparent affinity between the interacting partners. Surveys of different complexes involving IDPs suggest that there is a whole spectrum of different behaviours ranging from foldable IDP to extremely disordered, and unfoldable, systems [20].

From a thermodynamic perspective, Fuxreiter, Tompa and co-workers introduced a comprehensive concept, named 'fuzziness', which successfully captures the different behaviours recalled earlier [22–25]. A fuzzy complex is characterized by a structural heterogeneity, or multiplicity, which is critical in its function(s) [26]. Importantly, fuzziness should not be confused with the dynamics associated to the thermal motions experienced by a protein complex in a discrete thermodynamic well; a fuzzy complex is in fact characterized by the co-existence of several minima with similar free-energy content (Figure 1). The physiological relevance as well as the abundance and complexity of fuzziness have been extensively reviewed elsewhere [22,25,27,28] and will be briefly recalled later in the article, in the context of the observed mechanisms of binding-induced folding. In this context, however, it is worth emphasizing how the fine depiction of these structural ensembles under different conditions is critical to establish structure–function relationships using this formalism [29,30].

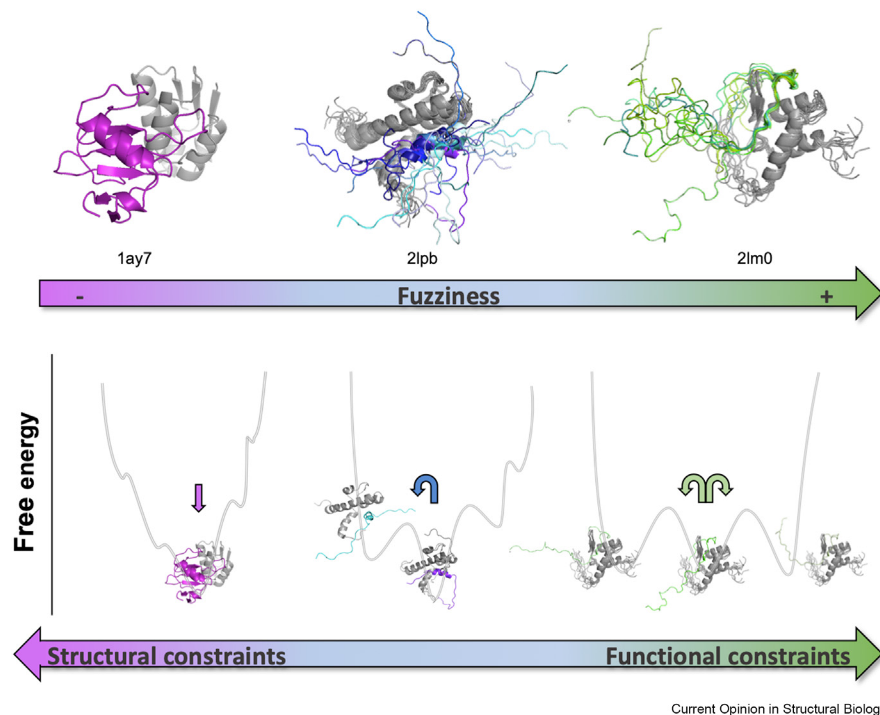
Binding kinetics and the order of events

As outlined earlier, the mechanism of recognition between IDPs and their physiological partners is expected to be a complex reaction that involves the productive encounter between the two partners, which is a bimolecular step, and the folding of the IDP system, a monomolecular reaction. In fact, as noted earlier, whilst the level of disorder in different complexes may vary considerably, it may be postulated that in all cases binding results in changes in the dynamic and structural behaviour of the IDP [20]. Therefore, a complete analysis of the kinetics of binding of IDPs requires i) to define the order of events in the reaction, that is, if folding precedes or follows binding, and ii) to provide a structural depiction of the relevant states.

At variance with the expected theoretical complexity of folding upon binding, the experimental characterization of several IDPs reveals a striking simplicity of the observed kinetics. In fact, several IDPs were found to conform to a simple two-state behaviour, showing single-exponential time courses and a linear dependence on reactant concentrations, another typical signature of two states [9,31–34]. Obviously, not all IDPs conform to a two-state reaction and, in some cases, at least one intermediate could be identified [35,36]. Nevertheless, experimental data collected so far suggest folding upon binding to be highly cooperative and only a limited number of highly elusive intermediates may be observed, an observation that parallels what was found in the case of folding of globular proteins. In fact, also in these cases, small single-domain proteins tend to fold via an all or none reaction.

Given these premises, it is of course extremely difficult to characterize the different steps that take place during induced folding. Furthermore, a particularly difficult task is to define if folding precedes or follows binding. One of the earlier studies addressing this question was contributed by Wright and coworkers, who investigated the coupled binding and folding mechanism of the IDP pKID to a folded domain, the KIX domain [19,37]. By employing NMR relaxation dispersion, it was observed that pKID first forms an encounter complex, followed by the accumulation of a partially folded complex, which is then locked in place by the population of the fully bound state [37]. This finding led the authors to put forward an induced-fit type of mechanism, where binding precedes folding. Aside from these sophisticated NMR approaches [38,39], the order of events in binding-induced folding has been also addressed on different protein systems using classical kinetic approaches based on stopped-flow and temperature jump techniques, in the case, for example, of ACTR/NCBD [35,40], c-Myb/KIX [33,41,42], PUMA/MCL-1 [9], HPV16 E7/Rb [43] and N_{TAIL}/XD [36,44–46]. In all cases, it appears that folding after binding is a likely event in interactions involving IDPs. However, more

Figure 1



Representative examples of different degrees of fuzziness in protein complexes. In magenta, Ribonuclease SA complex with Barstar (PDB code: 1ay7) — The ligand possesses a well-defined three-dimensional structure which is retained upon binding and populates a single-energy minimum. Blue, Complex of the central activation domain of Gcn4 with Gal11/med15 (blue, PDB code: 2lpb) — Gcn4 is an IDP that undergoes a disorder-to-order transition upon binding. The presence of multiple energy minima due to protein frustration allows the protein to assume different conformations upon binding. In green, AF4-AF9 complex (green, PDB code: 2lm0) — The protein complex is highly frustrated, with consequent formation of a fuzzy complex, in which the IDP does not acquire a unique well-defined structure. The energetic profile is rough, with many energetic minima that allows the IDP to assume different conformations and to be more sensitive to system perturbations.

complex pictures have been drawn by stabilizing selectively the ordered states of IDPs by introducing cosolvents, as exemplified by the usage of trifluoroethanol in the case of c-Myb and N_{TAIL} [36] or TMAO in the case of ACTR/NCBD [47], or by site-directed mutagenesis, as in the case of ACTR/NCBD [8], the p53 transactivation domain (TAD)/MDM2 [48] or in the case of c-Myb [38].

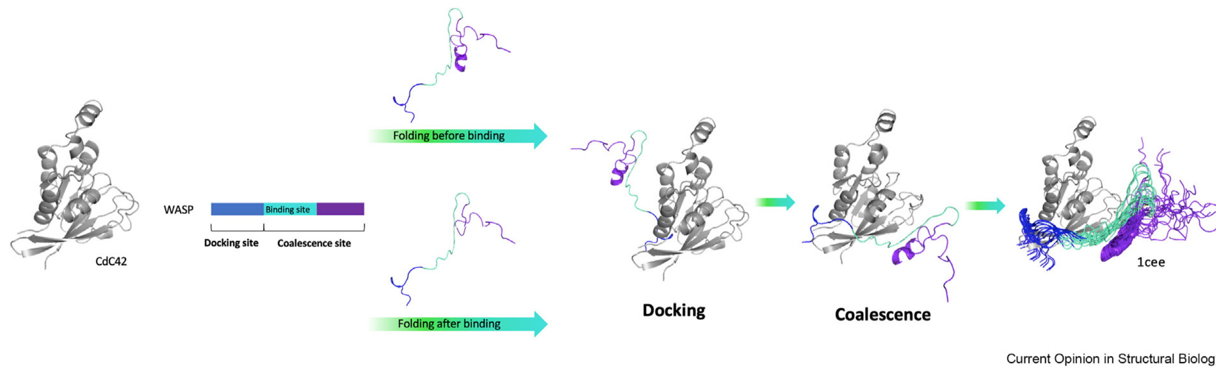
On the basis of the different experimental and theoretical work [49–52], we feel it is worth emphasizing that confining the mechanism of induced folding of IDPs to the classical induced fit or conformational selection scenarios might be simplistic. More likely, IDPs explore more complex mechanisms with multiple alternative pathways as suggested by the so-called dock and coalesce model [53,54] (Figure 2). Furthermore, the width of the conformational ensembles might depend on the relative propensity of a given IDP to explore preformed structure in the absence of its physiological binder. We note that this hypothesis parallels the slide between the so-called nucleation-condensation (highly cooperative and two-state) to diffusion-collision (framework with intermediates) mechanisms in globular protein folding, which is also tuned by the inherent stability of secondary structure elements within a given structure [55].

Protein engineering as a tool to understand IDPs

The most ambitious goal of the biophysicist is to provide a structural depiction of the sequence of events of a given reaction. In the case of binding-induced folding, such an issue is complicated by the highly cooperative nature of the reaction that, as recalled earlier, typically implies the presence of a limited number of transient intermediates. In this context, it has been proven very useful to apply an experimental methodology, known as the Φ value analysis, which was originally developed to study the folding of globular proteins [56] and has been subsequently extended to protein binding and induced folding [57].

The Φ value analysis is based on the assumption that a small structural perturbation, induced, for example, by conservative site-directed mutagenesis, has a little effect on the main reaction pathway. Under such conditions, by normalizing the effect of a given mutation

Figure 2



Dock and coalescence model as mechanism of IDPs binding proposed by Ou et al. [53]. The disordered region of WASP GBD folds upon binding and interacts with CdC42 GTPase (PDB code: 1cee). Two sequential steps characterize the dock and coalescence mechanism. In the first step, the docking region of the IDP WASP (in blue) interacts with CdC42 (in grey). In the second step, the rest of WASP protein (in cyan and purple) coalescences through additional intermolecular and intramolecular interactions. This mechanism is compatible with both conformational selection and induced fit models, thus enriching the frame of the events of the binding process of IDPs.

on the activation free energy versus the effect on the ground state, it is possible to map out interaction patterns in the transition state. In practice, a large number of site-directed mutants, insisting on different positions of the probed protein, are produced and expressed. Then, the kinetics of the reaction of each mutant is compared to that observed in the wild-type protein. The effect on binding kinetics is then compared to the effect on the binding affinity by following the formalism:

$$\Delta\Delta G_{D-TS} = RT \ln \frac{k_{on}}{k\prime_{on}}$$

$$\Delta\Delta G_{D-N} = RT \ln \frac{k_{on}}{k_{off}} \frac{k\prime_{on}}{k\prime_{off}}$$

$$\Phi = \frac{\Delta\Delta G_{D-TS}}{\Delta\Delta G_{D-N}}$$

where k_{on} and k_{off} denote the association and dissociation rate constants, respectively, and the symbol ‘ refers to a mutant protein.

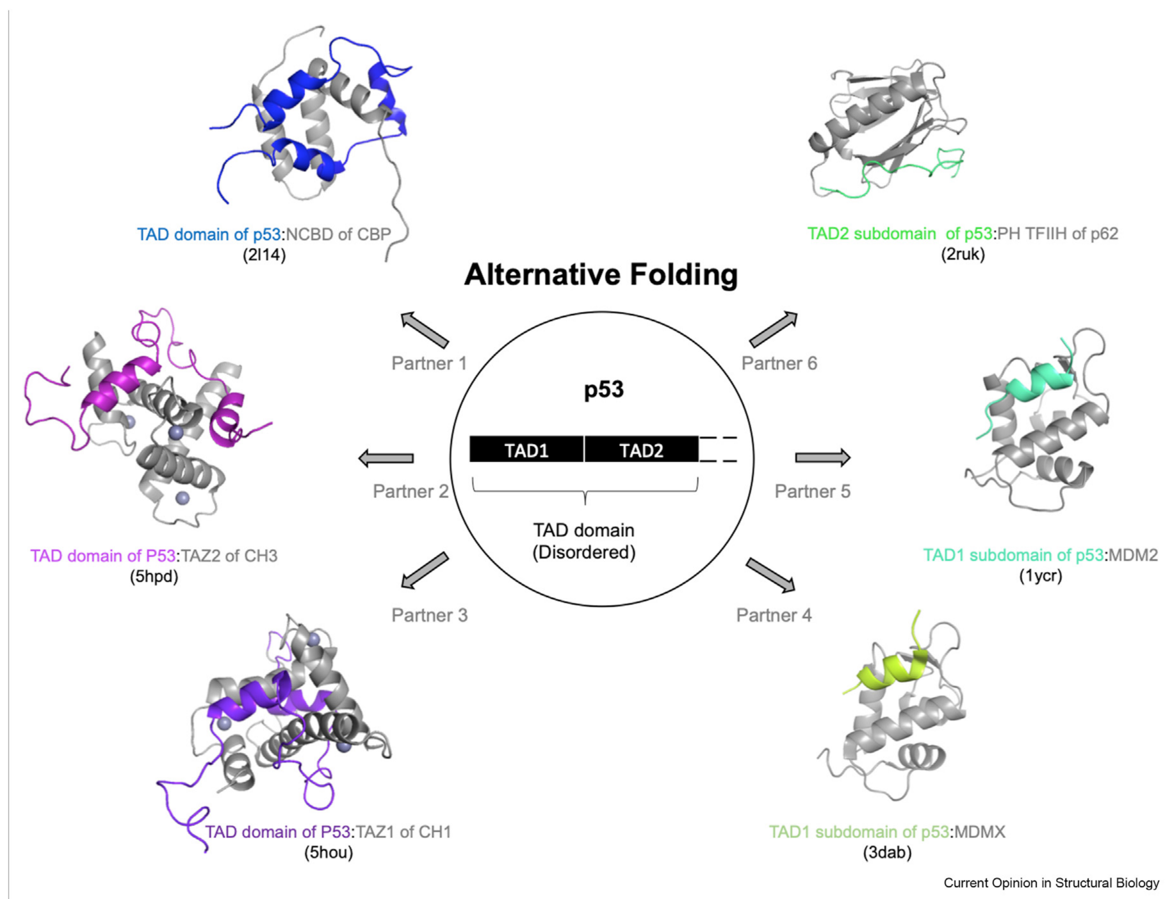
In the last decade, several IDP systems have been subjected to a complete Φ value analysis, providing important information about the induced folding reaction [8,44–46,58–61]. In particular, the analyses of several different IDPs have demonstrated that the transition state of induced folding resembles a distorted version of the ordered state, a finding that parallels what observed in the case of folding of globular domains. Of additional interest, however, close studies on the robustness of the reaction upon changes of experimental conditions have suggested this class of proteins to display malleable pathways that are directly influenced by their physiological partner [42,44,60,62,63]. In particular, since IDPs tend to fold via heterogeneous nucleation, whereby the transition state is directly stabilized by the interacting ligand, folding occurs via a ‘templated folding’ mechanism, whereby the structure of the transition state is dictated by the nature of the interacting partner [42,63]. Notably, the template folding mechanism represents a general mechanism, whereby multiple alternative partners can recognize the same IDP and induce cooperative folding. Thus, templated folding ensures the robustness of the cooperativity and at the same time increases the repertoire of different interaction partners, while minimizing aberrant interactions with undesired ligands [42,63,64] (Figure 3).

Frustration and emerging themes

One of the most elegant theories to describe the folding of globular proteins is founded on the principle of minimal frustration [65]. In a physical system, frustration occurs when each of the energetic interactions stabilizing the system cannot be simultaneously minimized by a single conformation. By following Wolynes, Onuchic and co-workers, the funnelled energy landscape theory postulates the presence of a strong energetic bias towards the native conformation [65]. Accordingly, the native state of globular proteins corresponds to a well-defined energy minimum where frustrated conflicts are largely absent. Natural proteins have been evolutionary sculpted by natural selection to be minimally frustrated.

Because proteins are evolved not only to fold but also to function, it was predicted that frustration patterns

Figure 3



Representative example of alternative folding of TAD1 and TAD2 subdomains of p53 are highlighted in blue (PDB code: 2l14), magenta (PDB code: 5hpd), purple (PDB code: 5hou), lime green (PDB, code: 3 dab), cyan (PDB code: 1ycr) and in green (PDB code:2ruk). TAD domain of the p53 protein is a prototypical IDP system that shows different folds upon binding with different partners. The folding pathway of TAD is thus consistent with a mechanism, whereby folding is templated by the structure of its ligands.

within the native state might structurally superpose with functional sites. Indeed, a survey of frustration in the PDB database confirmed this prediction and found frustration patterns to be located at the active site of enzymes, at the binding site of proteins forming complexes and even at allosteric sites of regulation [66,67]. Frustration could be calculated from the analysis of native structures and sequences, using an algorithm previously established [68].

By following these premises, we note that IDPs might be therefore considered as highly frustrated systems, where the contrasting demands between folding and function lead to disordered states. In fact, increasing frustration by suboptimal interactions also results in the presence of several energy minima and, therefore, the competition between several alternative structures [69], that is, disorder (Figure 1). Notably if such frustration is maintained in the bound state of an IDP, it may display a fuzzy behaviour. The joint consideration of fuzziness and frustration lead to a unifying framework, which can account for the interactions from structured to highly disordered proteins [26,64].

It might be of interest to consider some of the predictions arising from this view, on the light of the experimental data recorded on the mechanisms of binding-induced folding of IDPs. In fact, in the case of globular proteins, the funnelling of the landscape implies the sequence of the native state to be optimal for the native structure. Consequently, it is generally observed that site-directed mutagenesis results much more frequently in a destabilization, rather than stabilization, of the protein.

But what is the effect of mutagenesis on the folding step of an IDP? This question has been directly addressed on the induced folding reaction of the measles protein

NTAIL, an IDP system, and XD, its physiological partner. In fact, in this case, due to the complexity of the observed kinetics, it was possible to analyze independently the binding and folding steps. Remarkably, it was observed that of the different variants considered, only one destabilized the folding step of NTAIL, whereas the others showed either an increase in stability or a negligible change [44,45]. Hence, in line with predictions, it appears that in the case of fuzzy complexes and roughened energy landscape, the sequence is not necessarily optimized for a given structure and, therefore, sequence variations may induce a rearrangement of the conformational ensemble, rather than a destabilization. Moreover, it is also of particular interest to note that a recent analysis of the complex between the ETV/PEA3 family of activators and the coactivator Med25 demonstrated that small sequence variations within an activator family significantly redistribute the conformational ensemble of the complex while not affecting overall affinity [70]. These findings highlight the critical role of structural plasticity in the molecular recognition events mediated by IDPs and emphasize the need of additional studies to capture these effects in the context of the cellular environment.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was partly supported by grants from the Italian Ministero dell'Istruzione dell'Università e della Ricerca (Progetto di Interesse 'Invecchiamento' to S.G.), Sapienza University of Rome (RP11715C34AEAC9B and RM1181641C2C24B9, RM11916B414C897E to S.G), the Associazione Italiana per la Ricerca sul Cancro (Individual Grant – IG 24551 to S.G.), the Istituto Pasteur Italia (Teresa Ariaudo Research Project 2018, to A.T.) and European Union's Horizon 2020 research and Innovation programme under the Marie Skłodowska Curie Grant Agreement UBIMOTIF No 860517 (to S.G.). F.M. was supported by a fellowship from the FIRC - Associazione Italiana per la Ricerca sul Cancro (Filomena Todini fellowship).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Armstrong HE: **Poor Common Salt!** *Nature* 1927, **120**:478.
2. Bragg WL: **The structure of silicates**. *Nature* 1927, **120**: 410–414.
3. Perutz ML: **Stereochemistry of cooperative effects in haemoglobin**. *Nature* 1970, **228**:726–739.
4. Kendrew JC, Bodo G, Dintzis HM, Parrish RG, Wyckoff H, Phillips D: **A three-dimensional model of the myoglobin molecule obtained by x-ray analysis**. *Nature* 1958, **410**: 662–666.
5. Dunker AK, Silman I, Uversky VN, Sussman JL: **Function and structure of inherently disordered proteins**. *Curr Opin Struct Biol* 2008, **18**:756–764.
6. Chouard T: **Structural Biology: breaking the protein rules**. *Nature* 2011, **471**:151–153.
7. Uversky VN: **Unusual biophysics of intrinsically disordered proteins**. *Biochim Biophys Acta* 2012, **1834**:932–951.
8. Iešmantavičius 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.
9. 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* 2013, **136**:5197–5200.
10. Schuler B, Borgia A, Borgia MB, Heidarsson PO, Holmstrom ED, Nettels D, Sottini A: **Binding without folding - the biomolecular function of disordered polyelectrolyte complexes**. *Curr Opin Struct Biol* 2020, **60**:66–76.
11. Wright PE, Dyson HJ: **Intrinsically unstructured proteins: reassessing the protein structure-function paradigm**. *J Mol Biol* 1999, **293**.
12. Baronti L, Erales J, Habchi J, Felli IC, Pierattelli R, Longhi S: **Dynamics of the intrinsically disordered C-terminal domain of the nipah virus nucleoprotein and interaction with the x domain of the phosphoprotein as unveiled by NMR spectroscopy**. *Chembiochem* 2015, **16**:268–276.
13. Jensen MR, Communie G, Ribeiro EAJ, Martinez N, Desfosses A, Salmon L, Mollica L, Gabel F, Jamin M, Longhi S, *et al.*: **Intrinsic disorder in measles virus nucleocapsids**. *Proc Natl Acad Sci U S A* 2011, **108**:9839–9844.
14. Longhi S, Receveur-Bréchot V, Karlin D, Johansson K, Darbon H, Bhella D, Yeo R, Finet S, Canard B: **The C-terminal domain of the measles virus nucleoprotein is intrinsically disordered and folds upon binding to the C-terminal moiety of the phosphoprotein**. *J Biol Chem* 2003, **278**: 18638–18648.
15. Hibino E, Inoue R, Sugiyama M, Kuwahara J, Matsuzaki K, Hoshino M: **Interaction between intrinsically disordered regions in transcription factors Sp1 and TAF4**. *Protein Sci* 2016, **25**:2006–2017.
16. Mittag T, Orlicky S, Choy WY, Tang X, Lin H, Sicheri F, Kay LE, Tyers M, Forman-Kay JD: **Dynamic equilibrium engagement of a polyvalent ligand with a single-site receptor**. *Proc Natl Acad Sci U S A* 2008, **105**:17772–17777.
17. Sigalov AB: **Uncoupled binding and folding of immune signaling-related intrinsically disordered proteins**. *Prog Biophys Mol Biol* 2011, **106**:525–536.
18. Wu S, Wang D, Liu J, Feng Y, Weng J, Li Y, Gao X, Liu J, Wang W: **The dynamic multisite interactions between two intrinsically disordered proteins**. *Angew Chem, Int Ed Engl* 2017, **56**:7515–7519.
19. Dyson HJ, Wright PE: **Coupling of folding and binding for unstructured proteins**. *Curr Opin Struct Biol* 2002, **12**:54–60.
20. 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.
21. Borgia A, Borgia MB, Bugge K, Kissling VM, Heidarsson PO, Fernandes CB, Sottini A, Soranno A, Buholzer KJ, Nettels D, *et al.*: **Extreme disorder in an ultrahigh-affinity protein complex**. *Nature* 2018, **555**:61–66.
22. Fuxreiter M: **Fold or not to fold upon binding - does it really matter?** *Curr Opin Struct Biol* 2019, **54**:19–25.
23. Fuxreiter M, Simon I, Friedrich P, Tompa P: **Preformed structural elements feature in partner recognition by intrinsically unstructured proteins**. *J Mol Biol* 2004, **338**:1015–1026.
24. Miskei M, Horváth A, Vendruscolo M, Fuxreiter M: **Sequence-based determinants and prediction of fuzzy interactions in protein complexes**. *J Mol Biol* 2020, **432**:2289–2303. pii: S0022-2836(0020)30190-X.
25. Tompa P, Fuxreiter M: **Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions**. *Trends Biochem Sci* 2008, **33**:2–8.

26. Freiberger MI, Wolynes PG, Ferreiro DU, Fuxreiter M: **Frustration in fuzzy protein complexes leads to interaction versatility**. *J Phys Chem B* 2021, **125**:2513–2520.
•
The interaction between disordered proteins and their ligands are described by using the energy landscape theory, with emphasis on frustration. The paper demonstrates that local frustration occurring at the binding interface of IDPs complexes drives the possibility of populating different binding states characterized by specific frustration patterns. The study provides a quantitative description of how frustration and rough energy landscapes are correlated with specificity and versatility in IDP complexes

27. Fuxreiter M, Vendruscolo M: **Generic nature of the condensed states of proteins**. *Nat Cell Biol* 2021, **23**:587–594.

28. Fuxreiter M: **Classifying the binding modes of disordered proteins**. *Int J Mol Sci* 2020, **21**:8615.

29. Baldwin AJ, Kay LE: **NMR spectroscopy brings invisible protein states into focus**. *Nat Chem Biol* 2009, **5**:808–814.

30. Bonomi M, Vendruscolo M: **Determination of protein structural ensembles using cryo-electron microscopy**. *Curr Opin Struct Biol* 2019, **56**:37–45.
•
In this study the authors innovative view to employ CryoEM to understand and characterize protein disorder. In particular, the approach described by the authors uses CryoEM raw data, 2D class-averages and 3D density maps to characterize highly dynamic proteins or disordered portions of globular proteins and describe structural ensembles at high resolution. Different methods as well as caveats and pitfalls that may arise from data analysis are reported.

31. Åberg 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.

32. 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.

33. 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.

34. Narayanan R, Ganesh OK, Edison AS, Hagen SJ: **Kinetics of folding and binding of an intrinsically disordered protein: the inhibitor of yeast aspartic proteinase YPrA**. *J Am Chem Soc* 2008, **130**:11477–11485.

35. Dogan J, Schmidt T, Mu X, Engström Å, Jemth P: **Fast association and slow transitions in the interaction between two intrinsically disordered protein domains**. *J Biol Chem* 2012, **287**:34316–34324.

36. Dosnon M, Bonetti D, Morrone A, Erales J, di Silvio E, Longhi S, Gianni S: **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.

37. Sugase K, Dyson HJ, Wright PE: **Mechanism of coupled folding and binding of an intrinsically disordered protein**. *Nature* 2007, **447**:1021–1025.

38. 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.

39. Schneider R, Maurin D, Communie G, Kragelj J, Hansen DF, Ruigrok RW, Jensen MR, Blackledge M: **Visualizing the molecular recognition trajectory of an intrinsically disordered protein using multinuclear relaxation dispersion NMR**. *J Am Chem Soc* 2015, **137**:1220–1229.

40. Demarest SJ, Martinez-Yamout M, Chung J, Chen H, Xu W, Dyson HJ, Evans RM, Wright PE: **Mutual synergistic folding in recruitment of CBP/p300 by p160 nuclear receptor coactivators**. *Nature* 2002, **415**:549–553.

41. 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.

42. 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**:21994–22000.

43. Chemes LB, Sánchez IE, de Prat-Gay G: **Kinetic recognition of the retinoblastoma tumor suppressor by a specific protein target**. *J Mol Biol* 2011, **412**:267–284.

44. 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.

45. 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.

46. Troilo F, Bonetti D, Bignon C, Longhi S, Gianni S: **Understanding intramolecular crosstalk in an intrinsically disordered protein**. *ACS Chem Biol* 2019, **14**:337–341.

47. Dogan J, Toto A, Andersson E, Gianni S, Jemth P: **Activation barrier-limited folding and conformational sampling of a dynamic protein domain**. *Biochemistry* 2016, **55**:5289–5295.

48. Borcherds W, Theillet F-X, Katzer A, Finzel A, Mishall KM, Powell AT, Wu H, Manieri W, Dieterich C, Selenko P, *et al.*: **Disorder and residual helicity alter p53-Mdm2 binding affinity and signaling in cells**. *Nat Chem Biol* 2014, **10**:1000–1002.

49. Karlsson E, Paissoni C, Erkelens AM, Tehranizadeh ZA, Sorgenfrei FA, Andersson E, Ye W, Camilloni C, Jemth P: **Mapping the transition state for a binding reaction between ancient intrinsically disordered proteins**. *J Biol Chem* 2020, **295**:17698–17712.
•
This study provides an outstanding comparison between the mechanisms of recognition of an ancestral and a modern intrinsically disordered protein. It is shown that the coupled binding and folding mechanism is overall similar but with a higher degree of native hydrophobic contact formation in the transition state of the ancestral complex and more heterogeneous transient interactions

50. Jensen TMT, Bartling CRO, Karlsson OA, Åberg E, Haugaard-Kedström LM, Strømgaard K, Jemth P: **Molecular details of a coupled binding and folding reaction between the amyloid precursor protein and a folded domain**. *ACS Chem Biol* 2021, **16**:1191–1200.

51. Zosel F, Mercadante D, Nettels D, S B: **A proline switch explains kinetic heterogeneity in a coupled folding and binding reaction**. *Nat Commun* 2018, **9**:3332.

52. Robustelli P, Piana S, Shaw DE: **Mechanism of coupled folding-upon-binding of an intrinsically disordered protein**. *J Am Chem Soc* 2020, **142**:11092–11100.
••
In this paper the authors describe in atomic detail the binding induced folding reaction of NTAIL to XD. The study represents an extraordinary example of the power of unbiased molecular dynamics simulation using a super computer in describing the folding-upon binding event.

53. 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.

54. Zhou HX, Pang X, Lu C: **Rate constants and mechanisms of intrinsically disordered proteins binding to structured targets**. *Phys Chem. Chem. Pyhs.* 2012, **14**:10466–10476.

55. Gianni S, Guydosh NR, Khan F, Caldas TD, Mayor U, White GW, DeMarco ML, Daggett V, Fersht AR: **Unifying features in protein-folding mechanisms**. *Proc Natl Acad Sci U S A* 2003, **100**:13286–13291.

56. Fersht AR, Matouschek A, Serrano L: **The folding of an enzyme. I. Theory of protein engineering analysis of stability and pathway of protein folding**. *J Mol Biol* 1992, **224**:771–782.

57. Malagrinò F, Visconti L, Pagano L, Toto A, Troilo F, Gianni S: **Understanding the binding induced folding of intrinsically disordered proteins by protein engineering: caveats and pitfalls**. *Int J Mol Sci* 2020, **21**:3484.

58. Crabtree MD, Mendonça CATF, 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.

59. Dogan J, Mu X, Engström Å, Jemth P: **The transition state structure for coupled binding and folding of disordered protein domains**. *Sci Rep* 2013, **3**:2076.

60. • 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.
The authors demonstrate the heterogeneous structural nature of the transition state of folding upon binding using extensive site directed mutagenesis in synergy with molecular dynamics simulation.

61. Karlsson OA, Chi CN, Engström A, Jemth P: **The transition state of coupled folding and binding for a flexible β-finger**. *J Mol Biol* 2012, **417**:253–261.

62. 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.

63. Toto A, Malagrinò F, Visconti L, Troilo F, Pagano L, Brunori M, Jemth P, Gianni S: **Templated folding of intrinsically disordered proteins**. *J Biol Chem* 2020, **295**:6586–6593.

64. •• Gianni S, Freiberger MI, Jemth P, Ferreiro DU, Wolynes PG, Fuxreiter M: **Fuzziness and frustration in the energy landscape of protein folding, function, and assembly**. *Acc Chem Res* 2021, **54**:1251–1259.
A critical analysis of folding data on IDPs and globular proteins on the light of the concepts of fuzziness and frustration. A physical framework is presented, applicable to the structure and dynamics of proteins, opening up new perspectives for drug design involving highly dynamic protein assemblies.

65. Bryngelson JD, Onuchic JN, Socci ND, Wolynes PG: **Funnels, pathways, and the energy landscape of protein folding: a synthesis**. *Proteins* 1995, **21**:167–195.

66. Ferreiro DU, Hegler JA, Komives EA, Wolynes PG: **On the role of frustration in the energy landscapes of allosteric proteins**. *Proc Natl Acad Sci USA* 2011, **108**:3499–3503.

67. Ferreiro DU, Komives EA, Wolynes PG: **Frustration in biomolecules**. *Q Rev Biophys* 2014, **47**:285–363.

68. Ferreiro DU, Hegler JA, Komives EA, Wolynes PG: **Localizing frustration in native proteins and protein assemblies**. *Proc Natl Acad Sci U S A* 2007, **104**:19819–19824.

69. Gianni S, Camilloni C, Giri R, Toto A, Bonetti D, Morrone A, Sormanni P, Brunori M, Vendruscolo M: **Understanding the frustration arising from the competition between function, misfolding, and aggregation in a globular protein**. *Proc Natl Acad Sci USA* 2014, **111**:14141–14146.

70. •• Henley MJ, Linhares BM, Morgan BS, Cierpicki T, Fierke CA, Mapp AK: **Unexpected specificity within dynamic transcriptional protein-protein complexes**. *Proc Natl Acad Sci U S A* 2020, **117**:27346–27353.
In this paper, the binding reaction between ETV/PEA3 family of activators and the coactivator Med25 is characterized through stopped-flow kinetic binding experiments. Data carried out by the authors show that small sequence changes within ETV proteins resulted in major changes in the distribution of the conformational ensembles while not affecting the overall affinity of the complex, highlighting a key role of protein plasticity in mediating protein recognition.