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Current Opinion in Structural Biology

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

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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.

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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 complete overview of the section, please refer the article collection - Folding and Binding

Available online 11 December 2021

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

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].

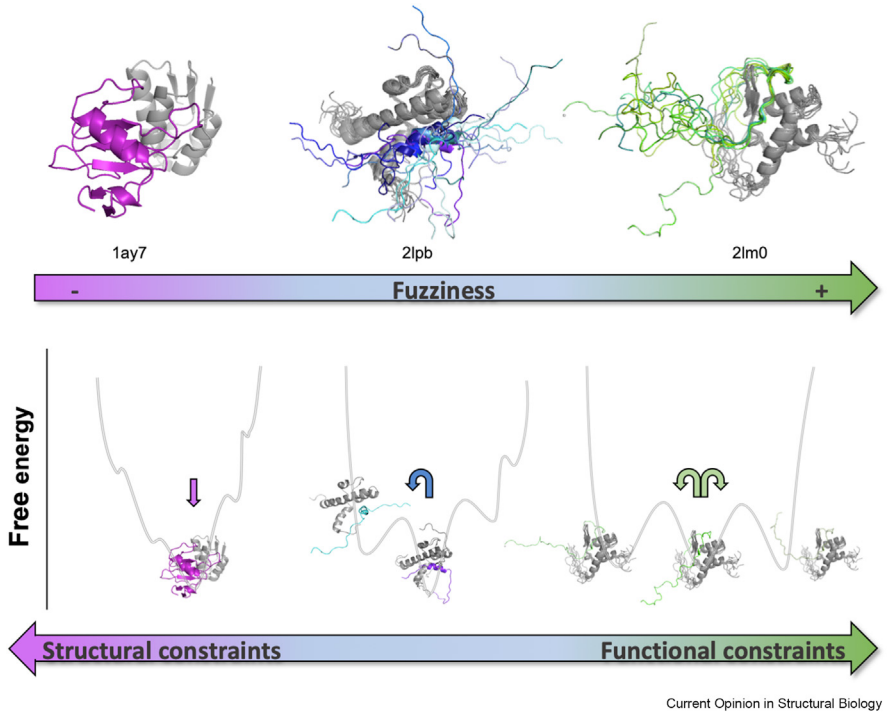
Binding kinetics and the order of events

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

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.

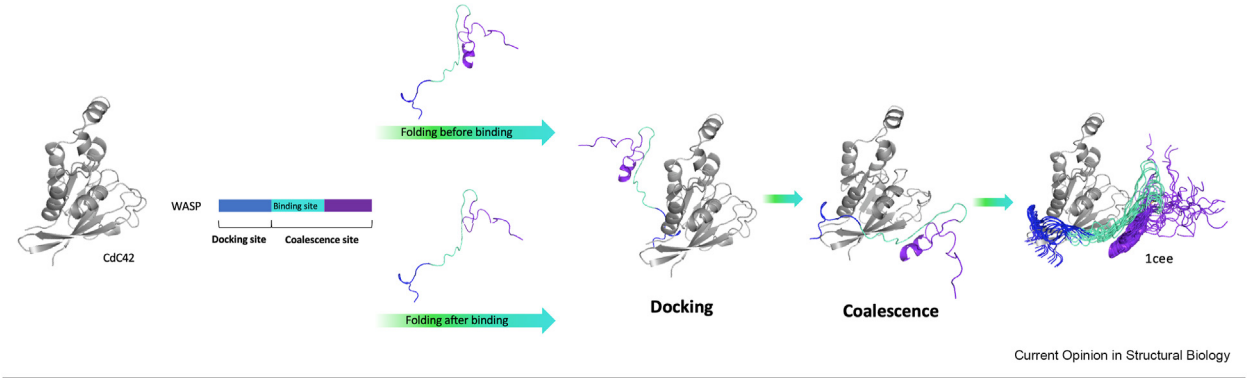
sophisticated NMR approaches [38,39], the order of events in binding-induced folding has been also addressed on different protein systems using classical kinetic approaches based on stopped-flow and temperature jump techniques, in the case, for example, of ACTR/NCBD [35,40], c-Myb/KIX [33,41,42], PUMA/MCL-1 [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 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 trans-activation 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

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.

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 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:

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

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

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

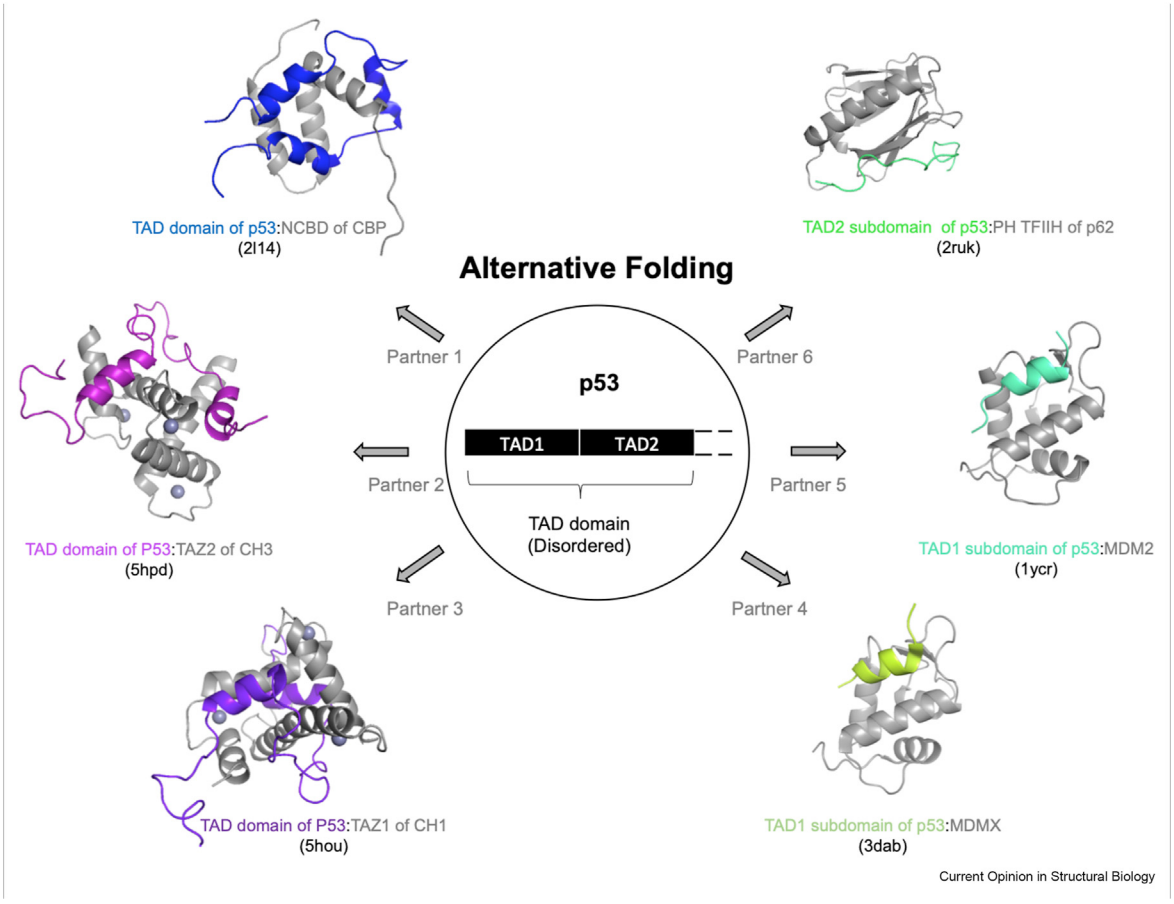
where k_{on} and k_{off} denote the association and dissociation rate constants, respectively, and the symbol ‘ refers to a mutant protein.

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

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.

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 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).

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