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Order/Disorder Transitions Upon Protein Binding: A Unifying Perspective

Olga O. Lebedenko[1] | Ashok Sekhar[2] | Nikolai R. Skrynnikov[1,3]

[1]Laboratory of Biomolecular NMR, St. Petersburg State University, St. Petersburg, Russia | [2]Molecular Biophysics Unit, Indian Institute of Science Bangalore, Bengaluru, India | [3]Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

Correspondence: Nikolai R. Skrynnikov (n.skrynnikov@spbu.ru)

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ABSTRACT

When two proteins bind to each other, this process is often accompanied by a change in their structural states (from disordered to ordered or vice versa). As it turns out, there are 10 distinct possibilities for such binding-related order/disorder transitions. Out of this number, seven scenarios have been experimentally observed, while another three remain hitherto unreported. As an example, we discuss the so-called mutual synergistic folding, whereby two disordered proteins come together to form a fully structured complex. Our bioinformatics analysis of the Protein Databank found potential new examples of this remarkable binding mechanism.

The realization that proteins are often intrinsically disordered has had a transformative effect on structural biology [1]. Although there are many flavors of disorder [2], for the purpose of this comment we use the binary classification assuming that the protein can be either ordered or disordered. Considering protein–protein binding, this dichotomy gives rise to some interesting combinatorics (see Figure 1).

Scheme **1** in Figure 1 illustrates the conventional type of binding when two structured proteins form a complex. Scheme **4** illustrates the more recently conceptualized "folding upon binding" process [4]. Binding of an intrinsically disordered protein (IDP) to its structured partner does not necessarily induce folding. The bound IDP can also remain disordered, giving rise to the so-called "fuzzy complex" [5], as shown in Scheme **5**.

Some other possibilities are less widely known, but have also been documented. The process termed "unfolding upon binding," see Scheme **2**, has been observed in chaperonin machines and protease machines, where the protein substrate gets unfolded for the purpose of subsequent refolding or shredding [6, 7]. Concerted folding/unfolding upon binding, such as shown in Scheme **6**, has also been observed. For instance, the interaction between disordered BH3 domain from pro-apoptotic protein PUMA with its anti-apoptotic counterpart BcL-xL falls in this category [8, 9]. While BH3 folds upon binding, BcL-xL undergoes (partial) unfolding. Furthermore, a pair of IDPs can form a complex while retaining their disordered character, as illustrated in Scheme **10** [10, 11]. There has been an early report of a specific homodimer involving cytoplasmic domain of the ζ chain from the T-cell receptor, which remains thoroughly disordered [10]. More recently, the tight complex between histone H1 and its nuclear chaperone prothymosin-α has been reported [11].

We will further discuss in somewhat greater detail Scheme **8**, where the coming together of the two IDPs leads to the formation of a structured complex. It is known that a folded protein can be reconstituted from its disjointed fragments, which are unstructured on their own [12]. This suggests that Pathway **8** should also be available to interacting IDPs. Of further note, the systems have been described where two disordered

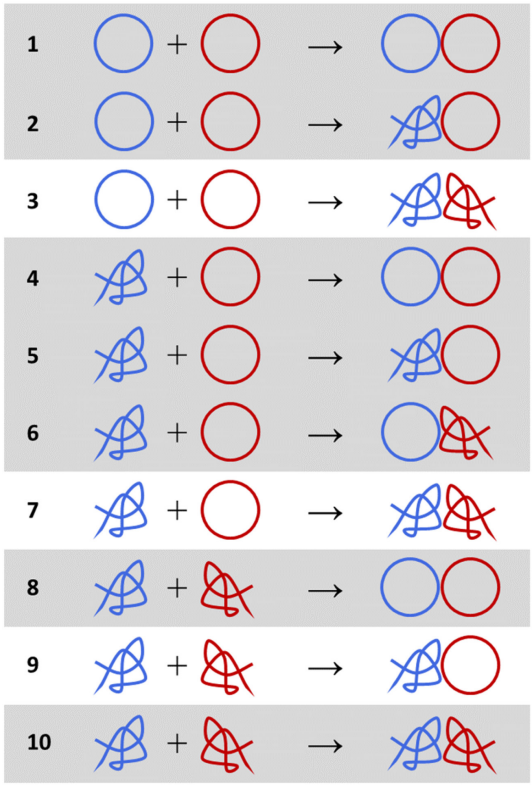

FIGURE 1 | Different variants of order–disorder transition upon protein binding. Those scenarios that have been observed experimentally are indicated by gray shading. The representation is limited in that it refers only to the initial and final states of the system and does not address the mechanism of binding. For example, Scheme **4** may involve conformational selection or induced fit or combination thereof [3].

proteins co-assemble to form bimolecular oligomers or fibrils [13, 14]. These observations likewise support the feasibility of Scheme **8**. One early example of such concerted disorder-to-order transition involves dimerization of the basic helix–loop–helix leucine zipper domains from transcription factors Fos and Jun [15], Myc and Max [16], and others. Later, a number of the so-called two-state homodimers (where the monomers are disordered but become structured upon dimerization) have been reported [17, 18].

Pathway **8**, as shown in Figure 1, was first conceptualized by Demarest et al. in their study of the complementary interaction domains of transcriptional coactivators CBP and ACTR [19]. While the two chains are disordered in isolation, they form a unique all-helical fold when bound to each other. This behavior has been termed "mutual synergistic folding". Later it emerged that the said CBP domain is, in fact, loosely structured, but can adopt different conformations upon ligand binding [20–22], which is a trademark IDP behavior.

Interestingly, a similar theme appears in the study of p53 core domain and its oncogenic mutants, some of which are highly unstable [23]. As one may expect, binding of a peptide ligand can successfully restore such destabilized domain to its native-like structured state [23]. The mechanism whereby binding of a linear IDP motif stabilizes an unstable (i.e., structurally variable) target can be exploited for therapeutic uses [24].

Other examples of Scheme **8** have been described by Leach, Kuntimaddi, and others, who investigated the interactions between the two components of the super-elongation complex, AF9 and AF4, as well as the interaction between AF9 and methyltransferase DOT1L [25, 26]. In these cases, the interacting segments are truly disordered, yet give rise to a fully structured complex. Of note, both of these studies, as well as the previous work by Demarest et al., rely on co-expression of the partner proteins to obtain a stable sample of the complex. In all cases, the structures of complexes **8** have been obtained by NMR spectroscopy, which is probably not accidental given their dynamic origins. At the time of their discovery, these complexes represented new, previously unobserved folds, which is likely a consequence of their unique folding mechanism.

Relatively recently, Fichó et al. undertook a search of the PDB database looking for binary protein complexes where there is experimental evidence that both components of the complex are disordered when studied in isolation [27]. This search led to a small MFIB database, comprising 49 complexes belonging to several representative classes. Since the experimental information on protein disorder is somewhat limited [28, 29], one possible alternative is to rely on sequence- and structure-based predictors. Previously, this type of strategy has been successfully employed to identify the examples of "folding upon binding" behavior, Scheme **4** [30, 31].

To this end, we have used one of the best-performing sequence-based disorder predictors, flDPnn [32, 33]. We have also employed heuristic structure-based NMA-NIA (normalized monomer area–normalized interface area) prediction method [31, 34]. The latter algorithm is based on the observation that IDPs that undergo "folding upon binding" tend to adopt extended conformations when forming a complex; as a consequence, they are characterized by relatively large per-residue surface area and per-residue interface area. Both flDPnn and NMA-NIA predictors have been applied to nearly 4500 unique heterodimers from the PDB database. In this manner, we have identified 44 candidate complexes that likely result from mutually synergistic folding (see Table S1 for details).

Among them is the complex with PDB identifier 5L85 that represents the interaction between human ZNHIT3 and NUFIP1 proteins (see Figure 2A) [35]. This interaction promotes the assembly of box C/D small nucleolar ribonucleoparticles. There is no direct experimental evidence that the two constituent chains of the complex are intrinsically disordered. However, their structures have never been obtained separately from each other. The structural study of the ZNHIT3–NUFIP1 complex (i) relies on co-expression of the two constructs, (ii) is conducted by means of solution NMR spectroscopy, and (iii) produces a structure, which represents a unique, previously unobserved fold [35]. All of this is similar to the known examples of complexes formed via Mechanism **8** (see above).

Very recently, the same group of investigators solved the structure of the ZNHIT3–NUFIP1 complex from *Plasmodium falciparum* [36]. In this case, the authors have made an attempt to express the two components of the complex separately. However, NUFIP1 peptide has been degraded during the bacterial expression. ZNHIT3, while expressed successfully,

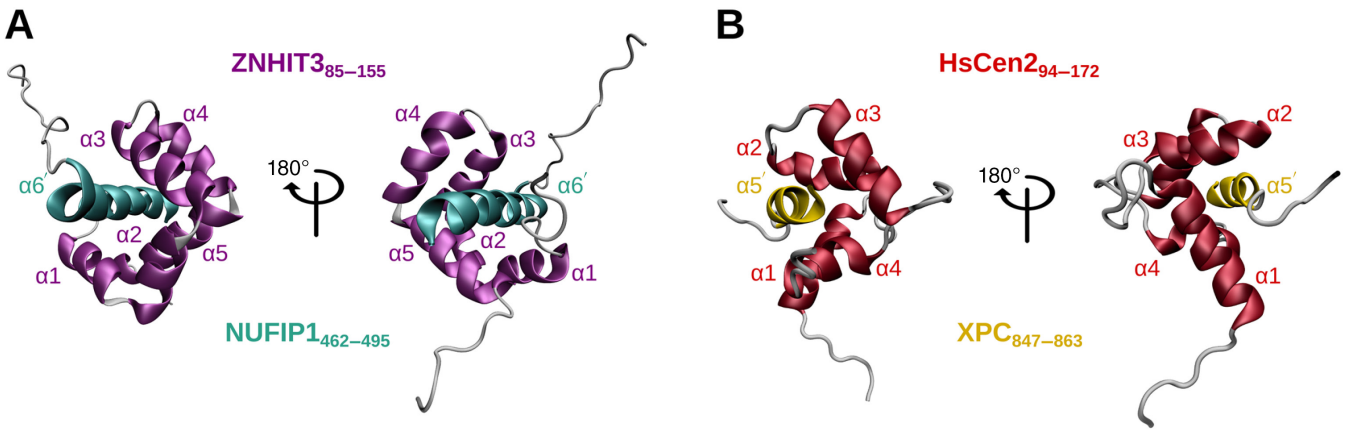

FIGURE 2 | (A) Structure of the complex between human NUFIP1$_{462–495}$ (turquoise) and ZNHIT3$_{85–155}$ (plum) as obtained by Quinternet et al. [35]. Shown is the first (lowest-energy) conformer from the NMR bundle PDB ID 5L85. (B) Structure of the complex between human HsCen2$_{94–172}$ (cherry red) and XPC$_{847–863}$ (gold) as obtained by Yang et al. [39]. Shown is the first (lowest-energy) conformer from the NMR bundle PDB ID 2A4J.

produced an extremely poor quality HSQC spectrum with a handful of stronger peaks and some weaker peaks positioned near 8 ppm in proton dimension; such spectral pattern is typical of molten globule proteins [37] (although it can also observed for structured proteins experiencing aggregation). Furthermore, the authors recorded an MD trajectory of ZNHIT3 in an *apo* form; for this purpose, they deleted the NUFIP1 helix from the structural model of the complex. The simulation led to an immediate "collapse" of the structure, i.e., closure of the helical ZNHIT3 clamp illustrated in Figure 2A. All of this evidence strongly suggests that both chains, in fact, lack any well-defined structure when studied in isolation and only become structured upon complex formation, in accordance with our bioinformatics predictions.

The complex of ZNHIT3–NUFIP1 was not a part of the original MFIB database. However, while this paper was in revision, the updated version of MFIB was released, which contained 79 heterodimeric structures including 5L85. Interestingly, this structure has been added to MFIB based on indirect evidence related to the homologous complex from *Saccharomyces cerevisiae* [38]. Therefore, our findings have been independently confirmed using a different line of approach.

Still, we identified several examples of mutual synergistic folding, which are not contained in either the original or the updated MFIB (see Table S1). Among those is the complex 2A4J that models the interaction between human centrin 2 (HsCen2) and xeroderma pigmentosum group C protein (XPC) (see Figure 2B) [39]. These two proteins are a part of the nucleotide excision repair (NER) system, where they are responsible for damage recognition during the global genome NER [40, 41]. In the case of 2A4J complex, there is direct NMR evidence that XPC$_{847–863}$ peptide is strongly disordered whereas the HsCen2$_{94–172}$ construct (corresponding to the C-terminal domain of HsCen2) is a molten globule [39]. Hence, this appears to be a new, previously unrecognized example of mutual synergistic folding.

Of note, the complex 2A4J has a similar topology to the previously discussed 5L85 (cf. Figure 2). In both cases, we observe an α-helical clamp-like structure closing on a single-helix peptide. However, the actual topologies are different, with HsCen2$_{94–172}$ resembling a Λ-shaped clamp and ZNHIT3$_{85–155}$ a Π-shaped clamp. Interestingly, the C-terminal domain of centrin 2 belongs to the EF-hand superfamily, also called calmodulin superfamily. The proteins in this superfamily are well known for their ability to bind peptides, with bound peptides adopting helical conformation (the binding is usually contingent on EF-hand proteins being saturated with Ca^{2+}, but not necessarily so [42]). Thus, one may argue that C-HsCen2 is an intrinsically unstable EF-hand domain, which is stabilized by loading its peptide ligand.

In conclusion, we return to the discussion of Figure 1, which enumerates different binding schemes accompanied by order/disorder transitions. Regarding the remaining scenarios **3**, **7**, and **9** (unshaded rows in the plot), we are unaware of any experimental reports describing such behavior. However, knowing nature's versatility, we expect that such themes will also be uncovered in future. The simple binary combinatorics illustrated in Figure 1 hardly does justice to an incredibly complex interplay between order and disorder, which involves ternary and higher-order complexes, allosteric coupling between multiple domains, competition between several disordered regions for a given binding site, and so on [43–45]. A dualistic view of order versus disorder is a simplification in itself. As many NMR studies have found, disordered sequences often exhibit some degree of transient structure [46]. Changes in the structural state of IDPs should be more appropriately described as a shift in the populations of disordered and structured states rather than a simple binary conversion [47]. Nevertheless, already the simplified binary classification outlined in this report offers an intriguing perspective on order–disorder variations associated with protein binding and predicts the existence of new, hitherto unobserved, binding modalities.

Author Contributions

Olga O. Lebedenko: conceptualization, investigation, methodology, visualization, writing – review and editing, software, formal analysis, data curation. **Ashok Sekhar:** writing – review and editing, conceptualization. **Nikolai R. Skrynnikov:** conceptualization, investigation, writing – original draft, visualization, methodology, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data generated by bioinformatics analyses are available in the Supporting Information of this article.

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Supporting Information

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