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Intrinsically disordered proteins: emerging interaction specialists

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Intrinsically disordered proteins or regions of proteins (IDPs/IDRs) most often function through protein–protein interactions, when they permanently or transiently bind partner molecules with diverse functional consequences. There is a rapid advance in our understanding of the ensuing functional modes, obtained from describing atomic details of individual complexes, proteome-wide studies of interactomes and characterizing loosely assembled hydrogels and tightly packed amyloids. Here we briefly survey the most important recent methodological developments and structural–functional observations, with the aim of increasing the general appreciation of IDPs/IDRs as 'interaction specialists'.

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Introduction

Ever since the first observations that certain proteins fulfill their biological function without adopting a stable three-dimensional structure, they have been on the center stage of structural–functional studies. These intrinsically disordered proteins or regions of proteins (IDPs/IDRs) abound in the proteome of all organisms and play key roles in regulatory and signaling processes of the cell [1,2]. Because they lack a fixed fold, they cannot possess enzymatic activity, and often function by binding to other partners (small molecules, DNA, RNA or proteins) [3••], and act as hub proteins in interaction networks [2]. Functional and evolutionary flexibility in protein–protein interaction networks are significantly enhanced by IDPs, as their interaction profile can be fine-tuned with alternative splicing, posttranslational modifications (PTMs) [4], which may result in allostery and promiscuity. For example, the allosteric modulation of the interaction of viral E1A protein with CREB binding protein and retinoblastoma protein was recently described [5•]; the advantages and disadvantages of promiscuous binding functions were also recently discussed [6]. As IDPs usually play key roles in these processes, they are also often implicated in pathological transformations of the cell leading to cancer [7].

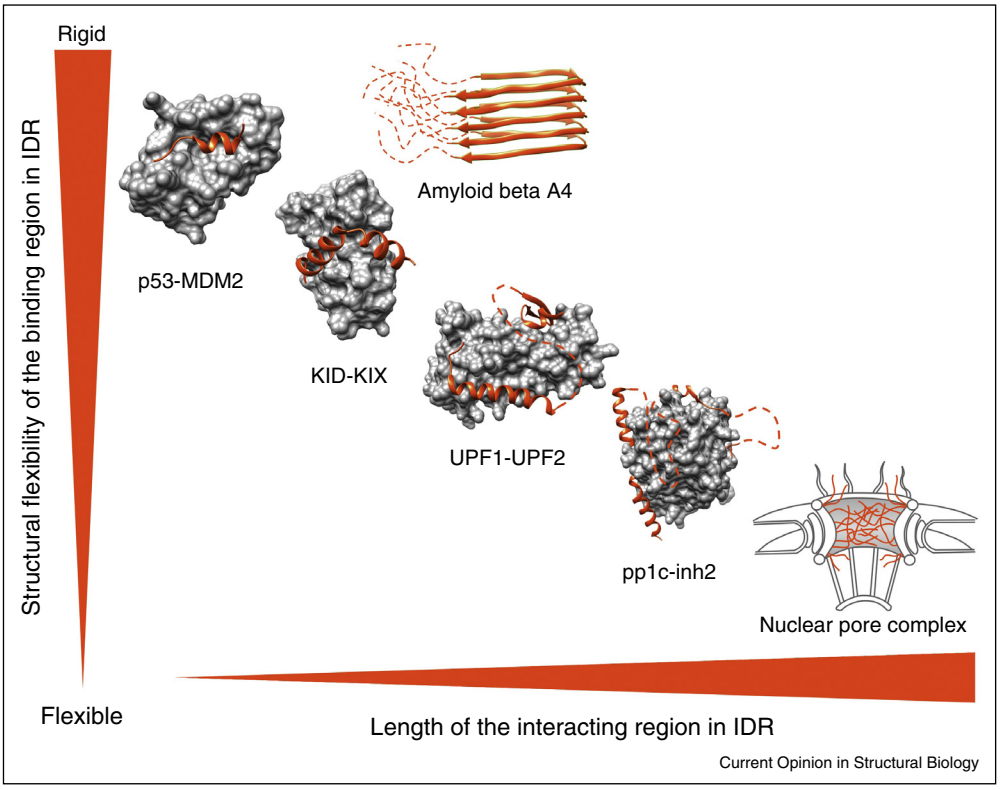
In the past few years there has been a tremendous advance in our understanding of the structural and functional subtleties of the different binding modes of IDPs (Figure 1). In this review we aim to walk the reader through the most recent discoveries in the field of IDP–protein interactions.

Motifs mediating the interactions of disordered proteins

One of the most enduring themes in IDP/IDR function is that their main functional elements are short recognition motifs (short linear motifs (SLiMs), or eukaryotic linear motifs (ELMs) [8] cf. Figure 2). Since the recent collection of proven motifs is only in the thousands [9] whereas their estimated number may be well above one million [10], we are very far from having a near complete map of interactions mediated by motifs. Experimentally verified interactions are deposited in PDB [11], and motifs are collected and categorized in the ELM database [9]. These snapshots about the bound form of the motifs provide valuable information on crucial residues, contacts, interactions, but usually have limited information on the mechanism, kinetics, dynamics and functional consequences of the interaction. As IDPs are highly flexible — they can be even disordered (fuzzy) — in the bound form, the detailed mapping of the mechanism of action is very important. In the last few years, we have made significant progress in the fine mapping and classification of interactions (Figure 1).

The binding mode of motifs is traditionally viewed in terms of folding upon binding (cf. Figure 1), when the protein undergoes a transition from a disordered to a bound, folded, state [12]. Recent results, however, tone this picture as binding of IDPs/IDRs may proceed by either conformational selection (when the bound conformation is pre-sampled in the disordered ensemble) or induced folding (also termed disorder-to-order transition,

Figure 1



Variability in structure and length within IDP interactions. Binding motifs of disordered proteins can form stable secondary structure upon binding, which can also be pre-formed in the free state of the protein (p53–MDM2 complex, PDB ID: 1YCR). A small flexible segment may interrupt rigid secondary structures inside the binding region of the IDRs, the orientation of the two helical structure and the flexibility of the turn region in the KID–KIX complex (PDB ID: 1KDX) can be further regulated by phosphorylation. In amyloid fibrils small disordered segments stacked (misfolded) to a very stable polymer usually in rigid β structure, other parts of the IDP remains flexible (picture based on 2BEG PDB structure). Long flexible linkers can also interrupt interacting regions enabling the binding on both sides of the globular partner (UPF1–UPF2 complex, based on PDB ID: 2WJV). Linkers between rigid binding regions can also participate in the binding, resulting in a 'fuzzy complex', where only small segment(s) will have positional preferences on the surface (pp1c–inh2 complex, based on PDB ID: 2O8G). At the other end of the flexibility scale, entropic chains create transient weak interactions, resulting a highly dynamic organic mesh (e.g. Nuclear pore complex, hydrogel). It is to be noted that the sharp distinction between structured and disordered regions in the complexes is not real and only reflects our simplification of structural reality: structured regions are highly dynamic and probably sample multiple (even unbound) states, and 'fuzzy' regions of residual disorder contribute to binding, that is, at least transiently interact with the partner protein.

when encounter with the partner initiates the folding process) [13–15], and even by induced unfolding (also termed order-to-disorder transition), when the disordered state arises upon complex formation (conditional or cryptic disorder [16••,17]). This structural mechanism may have several regulatory consequences, such as activation of the remaining part of the protein in an allosteric manner [18], releasing and exposing an interaction site for other partners [16••] or creating the active form of the protein [19].

In the more conventional, induced folding context, p53-MDM2 interaction was studied in great detail, both *in vitro* and *in vivo*. As reported first in Ref. [20], a single mutation in the MDM2 binding region of p53 protein transactivation domain can either increase (Pro27Ala) or decrease (Lys24Asn) the transient alpha-helix content of the binding motif [21•,22], without altering hydrophobic residues that mediate the interaction. It was also found that the structural propensity of the region before binding influences binding affinity, causing altered function *in vivo*. A similar correlation between structural propensity and binding affinity was observed by a series of mutations on the activation domain of ACTR, which can bind to the NCBD of CRE [13], suggesting that the preformed secondary structure is an important determinant of molecular recognition in IDPs/IDRs.

Regulation of the interaction by PTMs, such as multiple phosphorylation adjacent to the binding motif can also influence the binding potential and is an emerging theme in the IDP field. For example, in the PNT domain of