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The functional importance of structure in unstructured protein regions

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After two decades of research, intrinsically disordered regions (IDRs) are established as a widespread phenomenon. The growing understanding of the significant functional role of IDRs has challenged the structure–function paradigm, proving irrefutably that a stably folded structure is not a strict requirement for function. Nonetheless, (un)structure–function relationships remain at the core of IDR-mediated interactions. An IDR can populate a continuously transitioning continuum of structural conformations from fully disordered to stable globular states. In these ensembles, only subsets of conformations are binding competent, with *intramolecular* IDR contacts serving as important *intermolecular* binding determinants. Here, we review our current understanding of different types of intramolecular IDR interactions, their effects on IDR complex formation and their modes of biological regulation.

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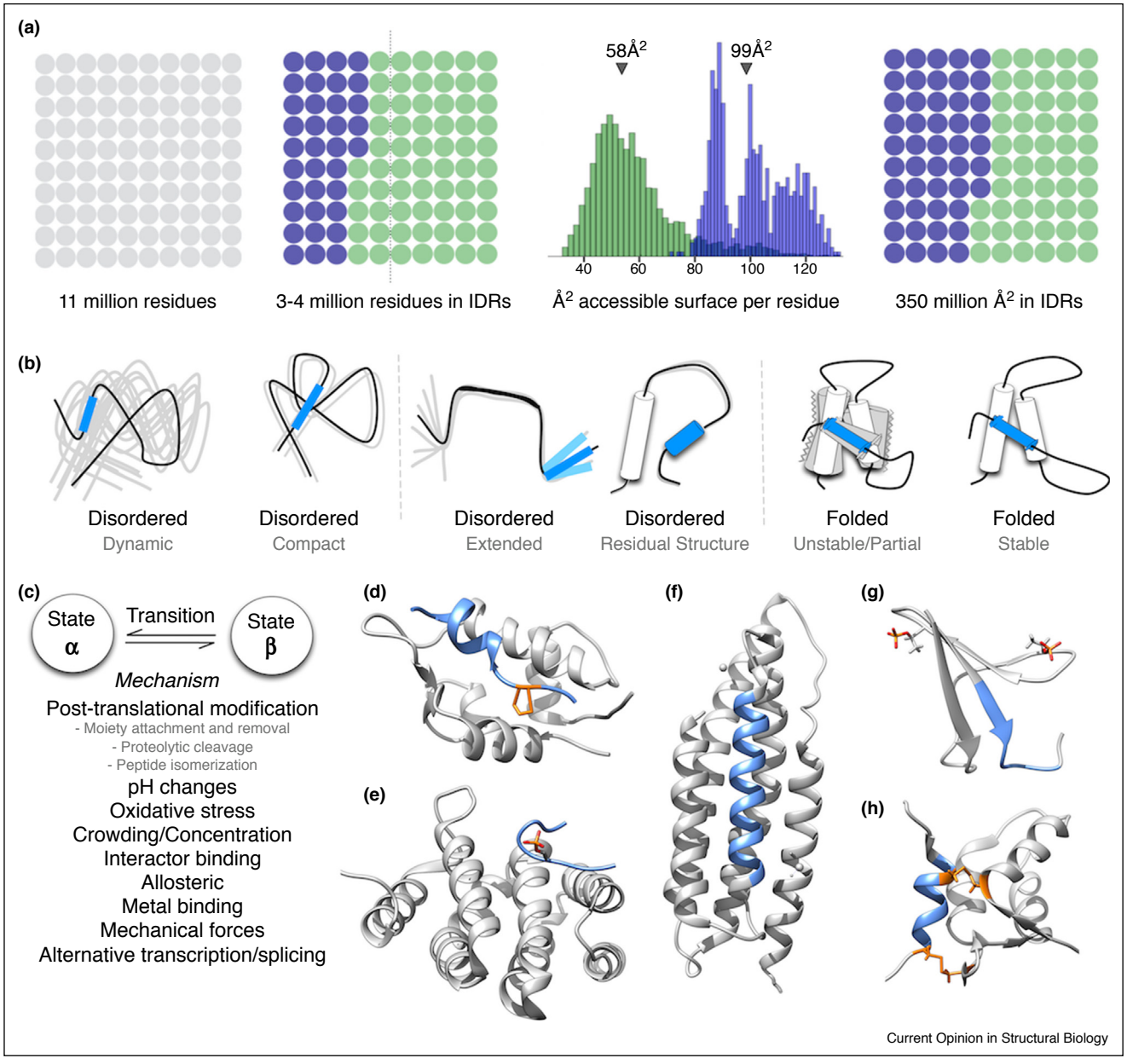
Introduction

Many proteins harbour extensive regions that lack stable secondary or tertiary structure in their native unbound states [1•,2,3••]. These protein segments, known as intrinsically disordered regions (IDRs), are predicted to constitute up to 40% of the residues in higher eukaryotic proteomes [4,5]. Given that many residues in the globular regions of the proteome are buried in inaccessible stabilising hydrophobic cores, up to half of the surface area of the human proteome accessible for protein interaction may be in unstructured regions (Figure 1a). Decades of research have uncovered the key functional roles of these regions in diverse biological systems. A key finding was the discovery that IDRs contain interaction modules, such as short linear motifs (SLiMs) and intrinsically disordered domains (IDDs), that mediated interactions and thereby confer functionality [6•,7•,8]. Estimates suggest that tens of thousands of interaction modules are encoded in the IDRs of the human proteome [9•]. These modules engage in diverse sets of activities that include providing enzyme docking sites regulating protein modification states, controlling protein stability (by recruiting ubiquitin ligases), acting as signals to target proteins to specific subcellular locations, directing dynamic complex formation or driving concentration-dependent phase transitions [2,6•,10]. Conversely, IDRs can contribute to protein function as a direct result of their structural properties without the requirement of a binding event, for example, by acting as flexible linkers or entropic chains [11,12•]. Finally, layers of transcriptional, post-transcriptional and post-translational regulation add cell type and cell state dependent conditionality to these IDR-encoded functions [13,14••,15,16].

Despite the growing acknowledgement of the key functional role of IDRs, the relationship between the structure and function of IDRs remains under-determined except for a few prototypic cases such as α-synuclein, tau, p53 and E1A. For historical reasons, our understanding of protein structure has been guided by our knowledge of stably folded domains. At the same time, the pervasive static linear representations of IDRs have restricted our perception of the structure and dynamism of IDRs. That is, we often think about IDRs as linear stretches of amino acids in two dimensions, and in isolation, rather than in three dimensions and in the context of crowded intracellular environments. As a consequence, the interplay between IDR structure and function is still poorly understood, especially in the context of the cell. Intrinsically disordered regions have unique compositional and biophysical properties that enable the sampling of a wide array of distinct conformations (Figure 1b) [17•]. In such ensembles, binding to IDR functional modules is only compatible with a subpopulation of the sampled conformational landscape [18•,19]. There are three key structural attributes that define these properties in representative terms: *transient structural elements*, *compact states* and *higher-order topologies*. Local, regional and global intramolecular contacts are at the heart of these three distinct structural attributes that can influence IDR function. Importantly, these structural attributes will often be conditional, integrating changes to cellular states into

Figure 1



(a) Of the ~11 000 000 residues of the ~21 000 primary isoforms of human proteins in the range of three to four million, or approximately one-third, of the residues are predicted to occur in IDRs (blue circles) [4,5]. On average, residues in IDRs have higher accessible surfaces area (blue, 99 Å^2 - based 667 models from 10 PEDB ensembles) than those in globular regions (green, 58 Å^2 - based on 2 738 CATH representative domain structures). Consequently, up to a half of the accessible surface area of the human may occur in IDRs. **(b)** Snapshots in the continuum of structural states of polypeptides. **(c)** Representative examples of mechanisms that can conditionally control the transition between the structural states of polypeptides. **(d)** A C-terminal proline (orange) modulates the helicity of the Mdm2-binding motif (blue) in p53 and thereby the binding affinity of the p53–Mdm2 interaction (PDB: 1YCR) [28••]. **(e)** The phosphorylated Ser2 of the CTD repeat of RNA polymerase (Pol) II (blue) does not contact the CID domain of the cleavage and polyadenylation factor Pcf1 but strongly enhances the affinity by stabilising the β-turn conformation of the CID domain-binding motif (blue) (PDB: 1SZA) [32,33]. **(f)** Mechanical unfolding of a five-helix bundle domain of talin reveals the vinculin-binding site (VBS) (blue) and results in the binding of the N-terminal vinculin head domain (PDB: 1SJ7) [43]. **(g)** Phosphorylation (orange) dependent folding of Eukaryotic translation initiation factor 4E-binding protein 2 (4E-BP2) results in a disorder-to-order transition and buries an eIF4E-binding YXXXXLΦ motif (blue) relieving 4E-BP2 dependent inhibition (PDB: 2MX4) [41••]. **(h)** Oxidation of Yeast AP-1-like transcription factor (yAP1) results in disulphide bond (orange) mediated folding of two distant regions into a four-helix bundle masking a nuclear export signal (NES) (blue) and resulting in cytoplasmic sequestration (PDB: 1SSE) [42••].

Box 1 How is the structure/function relationship of an IDR conditionally modulated?

Molecular switching mechanisms modulating SLiM or IDD function controlled by spatiotemporally conditional physicochemical, steric and competition-based switches are common in IDRs [14••,73–75]. A range of elegant mechanisms can also be hypothesised to conditionally modulate the structure and thereby the function of IDRs (Figure 1c). Every conformation sampled by an IDR can have distinct binding affinity and specificity properties for each binding partner. Therefore, cell state perturbations that regulate the conformational preferences to shift the populated conformations towards or away from binding-competent conformations will modulate the binding attributes of that region. From a conceptual point of view, this mode of regulation instigates a paradigm shift in our understanding of regulatory events as these cell state perturbations can control protein-protein interactions in an indirect manner without participating in the binding interfaces. Such modes of intramolecular communication bear all the hallmarks of disordered allostery [76]. Several examples of structural transitions of IDRs regulating protein functionality have already been characterised, for example, where a functional module becomes inaccessible through complete folding of a region [41••,42••,47•,77,78], or a required preformed secondary structural element for binding is promoted or inhibited [32,35•,36,79,80]. However, we have only begun to investigate the regulatory mechanisms controlling IDR function through IDR structure and the next decade will reveal a range of elegant and unexpected conditional structure–function relationships.

structural changes to regulate a protein's function (Figure 1c) (Box 1). In order to reassess the structure–function paradigm, the structural biology field must unravel the mechanistic principles that encode such intramolecular interactions and their effects on IDR functions. Furthermore, they must do so in a cellular context where additional contributions from physiologically relevant complex association and quinary interactions with cellular components may further exacerbate these structure–function relationships.

This perspective focuses on the structural modulation of the binding attributes of the functional modules commonly found in IDRs and highlights open questions in the field regarding the link between the structure and interactions of IDRs. In recent years, a new understanding of protein IDRs as key regulators of basic biological functions has emerged. It is vital that we discover the general design principles encoding the structural properties of IDRs and the effect of these structural properties on the function of IDRs. However, this will require new advances in key methodologies to comprehensively study these elusive biological phenomena [20•].

The contribution of structure to IDR-binding events

Local — transient structural elements

Most binding events involving IDRs necessitate transitions from dynamic unbound states to more constrained bound protein states [8]. Although, many of these interaction interfaces retain significant flexibility [21]. Furthermore, in some cases, high levels of dynamism can be observed even in the bound state [22••]. Approximately two-thirds of the functional IDR modules structurally solved in complex with their binding partners adopt defined secondary structures when bound [23]. In these cases, a preformed bound conformation can be a requirement for binding, a process known as conformational selection. Alternatively, the conformation can be adopted upon binding, a process known as induced fit. In many instances, both mechanisms contribute to the actual binding event [24,25,26•]. For several structurally characterised IDRs that interact with globular protein domains via disorder to order transitions, bound IDR states can be detected experimentally in free molecules as transiently populated, pre-structured motifs (PreSMo) [27•]. PreSMo's exert pronounced effects on actual binding behaviours because they reduce entropy costs associated with folding-upon-binding transitions [28••]. Therefore, the sequence of the functional module encodes both the physicochemical complementarity to the IDR-binding interface and the structural propensity of the region, and evolution can tune these attributes to optimise binding attributes [29,30•]. An elegant example to illustrate the contribution of transiently populated structures giving rise to defined binding affinities and productive biological outcomes is the p53–Mdm2 interaction (Figure 1d). By artificially increasing the levels of residual helicity within the IDR PreSMo of p53, the authors generated p53 mutants with enhanced Mdm2-binding affinities [28••,31]. Similarly, in the cell, the population of preformed secondary structural elements can also be modulated to strengthen or weaken IDR–ligand interactions in response to external and internal cellular cues (Figure 1c). For example, phosphorylation of the cleavage and polyadenylation factor Pcf1-binding motif within the CTD repeat of RNA polymerase (Pol) II does not form any intermolecular contacts but enhances binding affinity by stabilising the bound β-turn conformation [32,33] (Figure 1e). Another common example is the post-translational modification of the flanks of α-helices to positively or negatively regulate helicity and modulate binding [34,35•,36].

Regional — compact states

The accessibility of a functional module for recognition by a binding partner is a fundamental requirement of a biomolecular binding event. Non-uniform accessibility resulting from preferentially sampled compact disordered states are functionally intriguing. Compact collapsed states can be driven by local or long-range intramolecular contacts, with contributions from the physicochemical properties and flexibility of the polypeptide chain [17•,37•]. Compaction will influence the binding attributes to the modules within a region by limiting the accessibility of the functional modules. The functional role of constitutive collapsed disordered states has only

been studied in a handful of proteins. For example, α-synuclein where intramolecular contacts promote compact conformational states and shield the amyloidogenic NAC region [38••]. Interestingly, nitration of C-terminal tyrosines in α-synuclein has been shown to shift the population of sampled conformational states and this change can modulate binding of the N-terminus to membranes [39]. This example represents a mode of allosteric regulation that results not from the propagation of structural changes through a globular structure, as observed in classical allostery, but results from the modulation of the ensemble-averaged contribution of all sampled conformational states [40]. Most of the comprehensively characterised examples of a functional role of accessibility in IDR function involve large structural transitions and conditional folding (Figure 1f–h). The prototypic example is the phosphorylation-dependent folding of Eukaryotic translation initiation factor 4E-binding protein 2 (4E-BP2) that results in a disorder-to-order transition and buries an eIF4E-binding YxxxxLΦ motif relieving 4E-BP2-dependent inhibition [41••]. Several other similar examples exist and the diversity of the observed mechanisms suggests widespread usage of disorder-to-order transitions in the cell. For example, transition initiators ranging from oxidation, in the case of Yeast AP-1-like transcription factor (yAP1) resulting in disulphide bond mediated folding of two distant regions into a four-helix bundle masking a nuclear export signal (NES) [42••], to mechanical unfolding, in the case of talin to reveal the vinculin-binding motif and permitting binding of the N-terminal vinculin head domain [43], have been characterised. Several key questions remain unanswered: how accessible are the unstructured regions of the proteome *in vivo* [20•,44]? What proportion of these regions are partially or fully collapsed (compact globule or molten globule) [38••,45,46•]? Or even conditionally folded [19,41••,42••,47•,48]? How is the accessibility modulated by changes to cell state? And most importantly, how does the accessibility of the modules within these regions modulate their function?

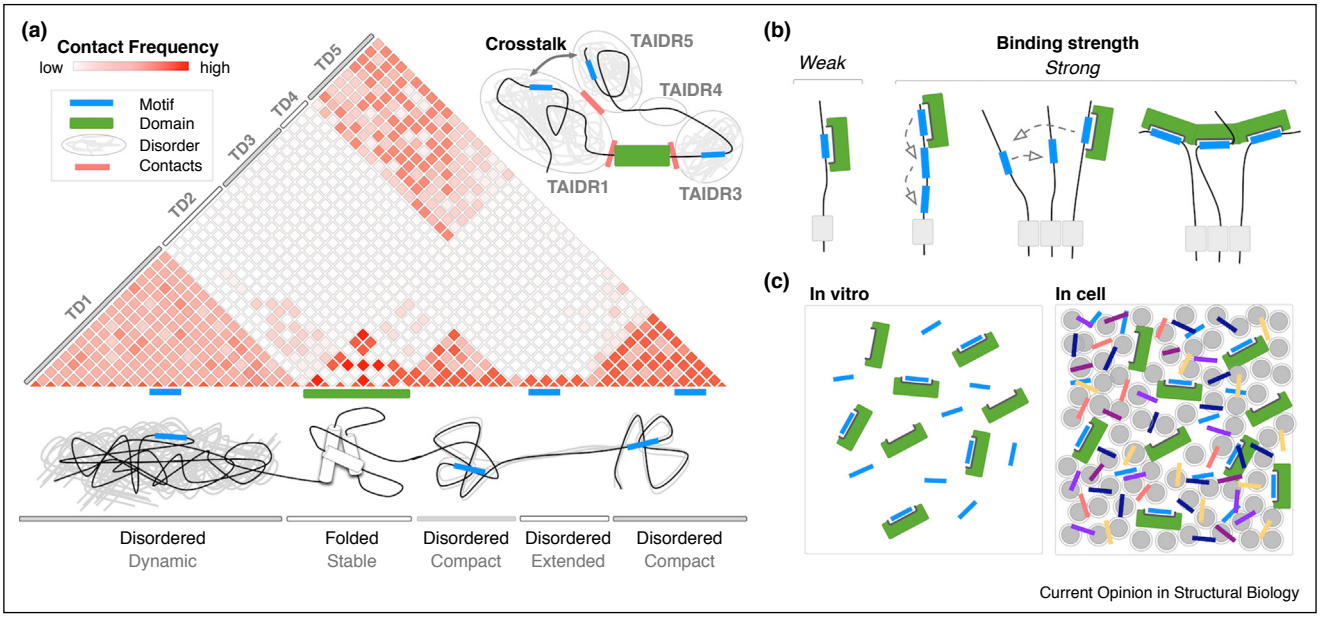
Global — higher-order structure

Many eukaryotic proteins are composed of multiple structurally independent segments organised by inter-region interactions to produce the topologies that define the super-tertiary structure of the protein [49]. The literature on higher-order structure of IDRs is sparse. Several IDRs have physiologically important auto-inhibited topologies that hide a functional module by intramolecular interaction with a globular region, for example, the Wiskott–Aldrich syndrome protein (WASP) [50•,51]. Additional basic cases exist such as the intramolecular interactions mediated by the N-terminal IDR of Colicin-N that protects the unstructured receptor binding region against proteolysis [52] or the organisation of the intrinsically disordered N-terminus of Src kinase around the SH3 domain [45]. These simple examples raise the question can multiple disordered regions in a protein or protein complex have complex higher-order topologies that modulates function [53]? Recent breakthroughs have shown chromatin in the nucleus has spatial organisation [54]. Distinct regions, known as topologically associating domain (TADs), preferentially co-localise in functionally significant higher-order chromatin structure. Analogously, long distance intramolecular interactions organising topology associated intrinsically disordered regions (TAIDRs) could result in preferential three-dimensional proximity of distinct disordered regions within a protein or complex (Figure 2a). This higher-order topology could act as a platform for signal integration by allowing the functional modules in these regions to cross-talk. Topologies of this type have been hypothesised for the Gab protein family [55•], but to date, no experimental evidence validating these hypotheses has been published. Hypothetically, the TAIDRs could be organised by numerous mechanisms including interaction with a globular region, disorder-disorder contacts or membrane anchoring and these mechanisms could be conditionally regulated to modulate protein function. This includes regulation by protein chaperones such as 14-3-3 or LC8 that could act as protein organisers analogous to the CCCTC-binding factor (CTCF)-dependent organisation of chromatin TADs [54,56,57].

Quaternary — IDRs in complexes

The low affinity of interactions mediated by functional modules in IDRs has long been a curiosity to molecular biologists. For example, the dissociation constants measured for isolated SLiMs interactions are usually in the low micromolar range [6•]. Many IDR-mediated interactions occur through multiple functional modules in a single IDR increasing module concentration and cooperatively producing dynamic yet strong interactions [58•,59,60]. The high local concentration of the functional modules or their binding partner will result in repeated binding–dissociation–rebinding events [61••]. Consequently, although the affinity of a single interaction is low, multiple IDRs or IDR-binding regions increases the on-rate of the binding and slows diffusion of the binding partners. The binding attributes of the complex can then be tuned by the number of these low-affinity interaction interfaces in the complex. This cooperativity is usually studied in a single IDR analysing avidity-based mechanisms, such as the ABBA-KEN-ABBA cassette of BubR1 binding to the APC/C holoenzyme [62], or allovency-based mechanisms, such as the phospho-dependent multi-motif-mediated interaction of TCOF1 and E3 Ub ligase CUL3-KBTBD8 [63•]. However, in the cell, many IDRs are present in multiple copies in large complexes. The complex association, oligomeric state or local concentration of the IDR-containing protein is often overlooked when we study protein interactions mediated by IDDs or SLiMs [64,65] (Figure 2b). Large quasi-stable

Figure 2



(a) Schema describing how preferential interactions mediated by Topology Associated Intrinsically Disordered Regions (TAIDRs) could drive higher-order super-tertiary structure of a protein. **(b)** Quaternary structure can modulate the binding attributes, and thereby function, of IDRs in multiple ways. Multiple motifs, multiple motif-binding pockets, or both in a single complex result in unique binding attributes that can be strong yet dynamic (green boxes denote motif-binding domains, blue blocks denote motifs, grey boxes denote globular domains, arrows denote binding–dissociation–rebinding events). **(c)** Quinary structure, the molecular crowding and interactions of the cell, represent a different environment to those commonly used in IDR experiments (green boxes denote motif-binding domains, blue blocks denote motif binding partner of green domain, non-blue blocks denote peptides with similar specificity determinants to the motif-binding partner of the green domain, grey circles denote molecular crowding by the cellular environment).

complexes can easily be built using co-operative functional modules within multiple IDRs and numerous key interactions in the cell utilise these design principles, for example, the high density of SH2 domain-binding motifs in large submembrane signalling complexes [61••], the repetitive IDRs driving phase transition [66], or the FG-repeat motifs in the nuclear pore [67]. Conversely, the multimerisation of the IDR-binding domain-containing protein can also be required for IDR binding. A canonical example is the trimerisation of TRAF2 required to bind three TRAF2-binding motifs in tumour necrosis factor receptor signalling [68,69] where a single motif-domain interaction is unable to produce a biologically relevant interaction [70]. In many cases, the oligomerisation of the IDR-containing or IDR-binding protein can be an important regulatory step, consequently, the system can use these properties to robustly encode a regulatory output [68].

Conclusion

This perspective posits that intramolecular contacts within IDRs will modulate the function of the interaction interfaces contained within these ubiquitous regions. These contacts will encode the transient secondary structure, accessibility and the higher-order structure of functional module-containing regions to contribute to their binding attributes, and thereby, function. The mechanisms controlling the structural properties of these regions will be both constitutive and non-constitutive. The structural properties of the constitutive examples will be directly encoded in the primary sequence of the IDR(s) and will fine-tune the binding attributes of the functional modules for their binding partner. Whereas the structural properties of the non-constitutive examples will be modulated in a cell state-dependent manner by external factors and act as conditional decision-making regions. Given the evolutionary plasticity of IDRs and the functional modules commonly found within them [29,30•,71], any regulatory mechanism controlling the function of a disordered region that is possible and accessible to evolution will likely exist.

The search for functional modules in IDRs has increasingly become the focus of intense research and the methodology for the functional analysis of disordered regions has reached the cusp of a high-throughput age [72]. Conversely, most of the structural mechanisms modulating IDR function are not easily accessible to the currently available structural biology methods. This suggests that the paucity of examples of structural

Box 2 Does a reductionist approach to studying protein interactions work for IDRs?

Do *in vitro* experimental findings on the structure–function relationship tell us what is happening in the cell [44]? If an isolated SLiM or IDD *in vitro* can function differently to the same functional module in the context of the complete IDR, the full-length protein, in a biologically relevant homomultimer or in complex, then how can we translate complex biophysical information gained *in vitro* to biological insight in the cell. Large IDRs can contain multiple subregions with distinct physicochemical, structural and functional properties and region, protein and complex will behave differently to fragments. Subregions may overlap and the conformations of one region may modulate the surrounding regions (Figure 2a). This presents an issue for experimental design where the boundaries or the oligomerisation state of the studied regions will alter the observed binding properties. Furthermore, as IDRs often contain multiple functional modules, an IDR can have multiple distinct bound states where given binding events can shift the population of conformations to a specific conformation, inhibiting or promoting binding at a distinct site from the interaction interface. For example, Mdm2 binding to P53 has been shown to induce long range interactions in the N-terminal IDR of P53 [81]. Finally, many IDRs are highly decorated with post-translational modifications that modulate the physicochemical properties of the region. Consequently, studying the physiologically relevant state of a protein is vitally important to understand the binding events of an IDR.

These experimental issues are compounded *in situ* as the quinary interactions of a protein differs significantly in solution, in cell extract and in the cell. Consequently, in the cell, both structural properties and protein-binding attributes of an IDR should diverge from the results of *in vitro* methods. The effect of the cellular environment on IDR structure has been tested for a handful of proteins [38••,46•]. However, the contribution of a heterogeneous crowded environment, physiological cell concentrations and competition from physicochemically similar peptides or surfaces similar to the IDR-binding pockets to IDR-mediated binding events has been largely neglected (Figure 2c). The effect of the cellular environment should contribute disproportionately to the binding attributes of lower affinity interactions, a hallmark of IDR-mediated interactions, than those of stable globular–globular interactions. Nonetheless, it is still unclear whether the binding attributes observed *in vitro* will be positively or negatively modulated in the cell [82]. It may be that there is no simple answer and the effect will depend on the composition of the IDR or the binding interface. Consequently, translating *in vitro* observations to in cell biological insight may be more complicated than we think. A key step is studying structural contributions to IDR-mediated interactions *in situ*; however, this is currently experimentally prohibitive [20•,83].

regulation of functional modules within IDRs is a reflection of the experimental difficulties characterising these mechanisms (Box 2). Consequently, it is important that we develop an *in vitro*, *in cell* and *in silico* framework capable of accessing the subtle yet functionally important contribution of the conformational space sampled by IDRs [20•]. With such a framework in place, we can comprehensively investigate the design principles encoding functional information in the dynamic interconverting conformations of IDRs. First, we need to take one simple step: we must start thinking about intrinsically disordered regions in three dimensions.

Conflict of interest statement

Nothing declared.

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