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IDPs in macromolecular complexes: the roles of multivalent interactions in diverse assemblies

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Abstract

Intrinsically disordered proteins (IDPs) have critical roles in a diverse array of cellular functions. Of relevance here is that they are components of macromolecular complexes, where their conformational flexibility helps mediate interactions with binding partners. IDPs often interact with their binding partners through short sequence motifs, commonly repeated within the disordered regions. As such, multivalent interactions are common for IDPs and their binding partners within macromolecular complexes. Here we discuss the importance of IDP multivalency in three very different macromolecular assemblies: biomolecular condensates, the nuclear pore, and the cytoskeleton.

Introduction

The study of protein biochemistry has long been guided by the paradigm that a protein's function is closely tied to its three-dimensional structure. However, over the past 15 years, there has been a growing appreciation that many proteins do not fit within the structure-function paradigm [1–3]. These proteins, called intrinsically disordered proteins (IDPs), lack tertiary contacts and typically do not exhibit stable secondary structure. Similarly, intrinsically disordered regions (IDRs) are relatively long (>30 residues) stretches of disordered regions which flank globular domains on one or both ends [4]. IDPs and IDRs have been identified as key players in many critical cellular processes [5] as well as being implicated in a diverse array of devastating diseases [6–8]. Of particular relevance here, IDPs are abundant, and play important roles, in the assembly and functions of macromolecular complexes [9,10].

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None.

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The characteristic features of IDPs make them ubiquitous in macromolecular complexes. IDPs are dynamic and flexible in solution; their extended conformations may permit them to 'search' efficiently for binding partners [11,12]. Their malleability allows them to adopt distinct conformations upon binding to different partners. As a consequence, the same IDP may be a component of several different complexes [13]. Moreover, IDPs are frequent targets for post-translational modifications (PTMs), which can tune their conformational states [14**,15] and interactions with binding partners [16]. Overviews of the types of interactions characteristic of IDPs can be found in several recent reviews [17,18].

IDPs typically interact with binding partners through short sequence motifs; short linear motifs (SLiMs), molecular recognition features (MoRFs), and low-complexity sequences (Box 1) [19–21]. Frequently two or more of these short motifs are found in IDPs or IDRs, making multivalent interactions common [19,21–24]. Multivalency arises when two or more binding sites are present on a protein allowing for simultaneous binding to partner molecules, increasing the overall avidity of the interaction (Box 1). It is important to a broad variety of biological interactions, including protein-carbohydrate interactions [25], clustering of cellular receptors [26], and virus-antibody binding [27]. Here we focus on the role of IDP/IDR multivalency in driving protein complex formation. Using three very different biological assemblies, we illustrate the diverse manifestations of multivalent interactions of disordered protein sequences.

Biomolecular condensates

The role of IDPs in biomolecular condensates (Box 1) is a topic which has garnered significant recent attention. Biomolecular condensates describe the micron-scale protein-rich assemblies resulting from liquid-liquid phase separation (Box 1) that are used by cells as a general mechanism for isolating internal material [28*–32]. These assemblies, which include nuclear bodies, Cajal bodies, P-bodies and granules, can play crucial roles in a variety of important biological processes [26,33–35]. They are differentiated from traditional organelles in that they lack a membrane barrier and therefore have the ability to exchange components in response to alterations to their environment. IDPs and IDR-containing proteins are enriched in many biomolecular condensates and have been reported to initiate phase separation both *in vitro* and *in cellulo* [36**–38]. In many of these cases, phase separation is driven by collective, weak, multivalent interactions between IDPs and/or their protein or nucleic acid partners [39]. PTMs to sequences involved can change the valency and intrinsic solubility of IDPs and thus tune their partitioning into liquid droplets [40*,41].

Phase separation driven by weak multivalent interactions may include heterologous electrostatic interactions between blocks of oppositely charged residues, either between proteins and nucleic acids or between mixtures of oppositely charged proteins (Figure 1, top right panel). The P granule protein LAF-1, thought to drive P-granule assembly *in vivo*, is an extensively studied example. The N-terminal disordered region of LAF-1 is an arginine/glycine (RGG) rich domain containing a mix of positively (arginine) and negatively (aspartic acid) charged residues. Electrostatic interactions between these alternatively charged regions results in its self-assembly into oligomeric structures and is necessary and sufficient for phase separation *in vitro* [36]. Additionally, the RGG domain of LAF-1 is important for

promoting dynamic protein-RNA interactions, shown to be critical in modulating droplet viscosity *in vitro* [36,42]. Multivalent interactions between LAF-1 and other P granule proteins, including PGL-1, PGL-3, and VBH-1, all of which contain oppositely charged disordered regions, are thought to underlie the maintenance of dynamic but coherent P granule structure [36].

A closely related example is the germ granule protein DEAD-box helicase 4 (DDX4), a RNA helicase with IDRs at both the N- and C- terminus [37]. DDX4 proteins are essential for the assembly and maintenance of the P-granules in *C. elegans*, and related assemblies in mammals and *Drosophila* [37]. Like LAF-1, the N-terminal IDR of DDX4 self-assembles and drives liquid droplet formation *in vitro*. It also contains RGG motifs as well as phenylalanine-glycine (FG) repeats which engage in cation-pi interactions with arginine residues within the RGG motifs, forming both intra- and intermolecular contacts [43]. DDX4 also provides an illustrative example as to how PTMs can alter interactions of IDPs; arginine methylation of DDX4 hinders its phase separation, likely due to decreased cation-pi interactions [37].

DDX4 and LAF-1 are examples of simple coacervation and do not require heterotypic interactions with a partner *in vitro*, although multiple additional partners are involved *in vivo*. In contrast, the disordered, negatively charged Nephhrin intracellular domain (NICD) requires multiple positively charged ligands/counterions to undergo phase separation [44**]. Although, NICD resembles DDX4 and LAF-1 in that it is highly charged and the charges are clustered into blocks, it has a high net negative charge. When overexpressed in HeLa cells, NICD forms nuclear liquid condensates with positively charged nuclear proteins serving as charge neutralizers [44**].

A variety of other IDRs driving liquid-liquid phase separation *in vitro* through multivalent interactions have been identified, including the amyotrophic lateral sclerosis (ALS) related and stress granule associated proteins, such as hnRNPA1, FUS, and TDP43 [38,45,46]. In contrast to DDX4 and LAF-1, FUS harbors mostly polar and aromatic residues and lacks charged residues. Mutational studies have shown that the aromatic residues are important for driving phase separation of FUS IDR, in particular through pi-pi stacking [45]. Notably all the multivalent interactions discussed here - aromatic, polar, and charge-charge – do not appear to induce stable canonical structure and are short lived, consistent with the dynamic nature of phase-separated droplets in cells [47].

Nuclear pore complex

Segregation of nuclear and cytoplasmic processes by the nuclear membrane is a hallmark of eukaryotic cells. The nuclear pore complex (NPC) is a large megadalton complex in the nuclear membrane that controls the exchange of materials between the two compartments. Small molecules of less than ~5nm in size can diffuse freely across the NPC while larger complexes are actively transported by the action of nuclear transport factors called Karyopherins (Kaps) [48]. IDPs and IDRs are an integral part of the NPC and they have multiple roles in the scaffolding and function of the NPC (Figure 1, bottom panel).

The NPC is made up of multiple copies of ~30 nucleoporins (Nups). Scaffold Nups are nucleoporins that make up the core ring structure of the complex. While many scaffold Nups use folded domains to interact with one another, certain scaffold Nups, often referred to as linker Nups, have SLiMs containing long IDRs that act as assemblers to bridge interactions between and within different subcomplexes [49*–51]. For instance, SLiMs in IDRs of Nup53 and Nup145N were found to interact with different scaffold Nups. Some of these interactions are mutually exclusive to one another, making it possible for linker Nups to drive the formation of distinct subcomplexes [49*]. This type of multivalent interactions is in contrast with those found in biomolecular condensates, as binding motifs are not found in tandem repeat. Instead different SLiMs are present binding different specific partners to form a defined scaffold.

More complex multivalent scaffolding interactions involving IDPs can also be found in the NPC. For instance, Nup159 of the Nup82 subcomplex, located on the cytoplasmic side of the NPC, contains a dynein light chain–interacting domain (DID) linked to a self-dimerizing helical region H1 [52*]. The Nup159 DID is intrinsically disordered and contains five short glutamine-threonine (QT) motifs that are recognized by the dynein light chain (Dyn2) homodimer [53–55]. Therefore, five Dyn2 dimers can bind to the Nup159 dimer concurrently and form a polybivalent duplex that has a rigid rod-like structure [10,54,55]. The use of multiple binding motifs and different dimerization mechanisms enhances the structural stability of the Nup159 dimer, which was found to be the main structural scaffold of the Nup82 subcomplex [52*,55].

IDPs are also critical for the function of the NPC as a selective barrier. The central channel is made up of FG-Nups. These are nucleoporins with long IDRs containing FG repeats in different combinations, such as FxFG or GLFG, that form various intra- and intermolecular interactions [56]. There is an ongoing debate regarding the details of how the FG repeats make up the selectivity barrier. Proposed models include selective phase, entropic polymer brush and Kap-centric models (reviewed in [57–61]). While these models disagree on the structure and properties of the FG-Nup barrier, it is well established that the passage of Kap-cargo complexes through the FG barrier is dependent on their interaction with FG repeats. Recent studies have illustrated that the Kap-FG interactions are weak, fast-exchanging and multivalent, which enables efficient transport across the FG-barrier (reviewed in [62**]).

The cytoskeleton

The cytoskeleton is a dynamic and complex network of filaments and associated proteins found throughout the cell cytoplasm. It is responsible both for providing a rigid internal scaffold, as well as being crucial for cell movement and changes in shape. As such, the cytoskeleton is an inherently dynamic assembly, able to grow and shrink in response to cellular signals, a process highly regulated by binding partners. Many of the cytoskeleton binding proteins are IDPs or have IDRs [63], and the critical dynamic growth feature of these filamentous complexes is dependent upon the disordered partners (Figure 1, top left panel).

Microtubules are the largest cytoskeleton filaments, composed of subunits of α/β tubulin heterodimers. Both α - and β -tubulin contain an N-terminal nucleotide binding domain and terminate in a highly acidic, unstructured C-terminal tail. The exterior of the microtubule is covered with these C-terminal tails which provide a multivalent binding surface for various motor and non-motor microtubule associated proteins (MAPs) [64**]. Moreover, tubulin C-terminal tails are the primary sites of PTMs which change the physico-chemical features of the tails. The PTMs are not evenly distributed throughout the microtubule surface, an observation that led to the hypothesis that a specific PTM pattern may form a 'tubulin code' which signals for specific binding of different microtubule binding partners [65]. For example, CLIP170 and P150 Glued, both of which contain long disordered regions, only bind to the tips of growing microtubules in conjunction with the protein EB1 and the presence of tyrosinated tubulin [66].

Non-motor MAPs, or structural MAPS, promote microtubule assembly and stability; there are two major families, Type I (MAP1) and Type II (tau, MAP2 and MAP4). There is relatively little structural information about Type I MAPS, although they are predicted to be largely disordered [67*]. Their highly basic N-terminal microtubule binding domains contain many short KKE motifs which mediate binding to the negatively charged outer surface of the microtubule [68]. Tau and MAP2C, both of which are neuronal proteins but differ in their cellular localization, are entirely disordered in solution [69]. They share a high degree of sequence homology in their microtubule binding domains which contain KxGS motifs important for microtubule binding [70]. Tau and MAP2C are thought to utilize multiple of these motifs for binding along the surface of microtubules, enhancing stability through bridging tubulin interfaces [69]. Recent work shows that tau also binds to soluble tubulin heterodimers with high affinity [71,72]. Tau utilizes multiple tubulin binding sites to form a 'fuzzy complex', characterized by the high degree of disorder even upon binding [73] to promote tubulin polymerization [74,75]. Another group of microtubule binding proteins with disordered regions, +TIP proteins, are classified by their accumulation at the growing end of microtubules. This is a diverse group of proteins which includes EB1, CLIP170 and P150 Glued, mentioned above [76]. Despite their structural, sequence and functional diversity, many +TIP proteins share a common motif important for localization to the microtubule end, which is one or more SxIP motifs embedded within a basic and serine/proline rich IDR [77].

Microfilaments consist of double stranded helical actin filaments made up of soluble actin monomer subunits. Because actin polymerization is kinetically unfavorable, cells rely on nucleation/elongation factors to regulate the process. Nearly all nucleating/elongating proteins (with the exception of formins, see below) utilize disordered WASP-homology 2 (WH2) actin binding motifs [78], with more than 800 proteins predicted to contain WH2 motifs [79]. The motif is primarily identified by a consensus actin-binding sequence, LKKT/V; also containing an N-terminal region which forms a short helix upon binding to actin [78]. In many nucleating proteins, the WH2 motif occurs in tandem repeats, giving rise to binding of multiple actin monomers. For example, Spire, a nucleation factor with four tandem WH2 domains, is thought to function by aligning actin monomers to favor actin assembly [80,81]. Intriguingly, actin regulatory proteins containing WH2 motifs have been

shown to phase-separate *in vitro* through multivalent interactions with other actin regulatory proteins [40*].

Formins are multidomain proteins, containing several structured N-terminal domains and a globular C-terminal formin homology 2 (FH2) domain linked by a proline-rich IDR (FH1) [82]. The FH2 domain has very low affinity for monomer actin and is thus thought to bind to small actin polymerization intermediates [83]. While the FH1 domains are not required for function, they greatly enhance the rate of actin polymerization over diffusion-limited rates. The polyproline segments, which bind to profilin, an actin regulatory protein, are joined by flexible linkers which allow for efficient search for profilin-monomer actin complexes [84]. Binding of one or more profilin-monomer actin complexes increases the effective local concentration of monomer actin near the FH2 domain, accelerating elongation [84]. FH1 domains contain between 2 and 8 proline rich segments, which individually display weak affinities (~5–1000 μ M) for profilin-monomer actin [63,83]. A study of yeast formins found that the elongation rate was positively correlated with the total number of polyproline binding sites in the FH1 domain [85]. Binding sites closer to the FH2 domain ‘search’ less space before delivery of the monomer actin to the FH2 domain and generally have lower affinity than those further away [83]. Thus, diverse enhancement rates are achieved by different formins through variation in both the number, and the affinity, of polyproline binding sites.

Conclusions

The number and diversity of IDPs are astounding; an estimated 40% of the human proteome contains significant stretches of disorder. While these proteins are involved in a diverse array of interactions, this review focuses on the role of multivalent interactions of IDPs in protein assemblies. The assemblies selected for this review exhibit varying levels of stability, ranging from relatively stable structures such as the NPC that enable regulated exchange of components between the nucleus and cytoplasm; to the dynamic cytoskeleton network, important for supporting overall shape and internal organization of the cell; to biomolecular condensates whose dynamics can be tuned in response to cellular stimuli. In each of these assemblies, repeats of short recognition sequences or self-assembly motifs present in disordered regions enable multivalent inter- and intramolecular interactions to form complex macromolecular structures. While such short sequences are critical for interactions involving IDPs, poor sequence conservation makes exact identification and delineation challenging, and remains an area of active research [21,86]. Coupled to improving methods for identifying interaction motifs is the need to further understand how physico-chemical properties of IDPs, other than the relatively well-characterized sequence motifs, affect multivalent interactions. For example, spacing between motifs and flexibility of linker regions may critically alter interactions with binding partners and consequently the formation of the macromolecular assemblies [87]. Furthermore, there is growing evidence that interactions between IDPs/IDRs and macromolecular binding partners are feasible and attractive targets for drug development [5,88–90]. Both identifying and understanding the molecular details of multivalent interactions driven by these short motifs within IDPs is an important area for future work.

Box 1

Short Linear Motifs (SLiMs) – Short stretches of sequences (3 – 10 residues) that direct protein-protein interactions.

Molecular Recognition Features (MoRFs) – Intrinsically disordered regions of 10–40 residues which undergo a disorder to order transition upon binding to protein partners.

Low-complexity sequences – Sequences that contain repeats of single or short amino acid motifs, with less diversity in amino acids than most protein sequences. While many low-complexity sequences are of unknown function, some modulate protein-protein and protein-nucleic acid interactions.

Multivalency – Interactions between two or more binding sites on a protein with its binding partners. Achieved through having different types of binding sites interacting with different macromolecules, or repeats of the same binding sites interacting with one or more binding partner.

Liquid-liquid phase separation – Demixing of a liquid phase from another, driven by energetically favorable interactions between components of different phases. One familiar example would be the separation of oil and water.

Biomolecular condensates – Biomolecular condensates, also termed as pleiomorphic ensembles, liquid droplets or membraneless organelles, are compartments in the cell formed via liquid-liquid phase separation. Their formation is driven by interactions between molecules which are enriched in the phase separated droplets. These compartments are liquid-like as they can fuse with one another, are dynamic and can undergo rapid exchange with the surrounding environment.

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Highlights

1. Intrinsically disordered proteins have diverse roles in macromolecular complexes.
2. Short sequences, repeated within disordered regions give rise to multivalent binding sites.
3. Multivalency allows disordered proteins to mediate formation of stable and dynamic complexes.

Figure 1. Multivalent IDP interactions diversify the application of disorder in macromolecular complexes

Top right panel: Biomolecular condensates. Weak transient interactions between multivalent proteins harboring IDRs drive liquid-liquid phase separation. a) Patterned electrostatic interactions between oppositely charged tracts of a single molecular species, as in LAF-1 and DDX4. Many RNA-binding proteins have modular architectures in which different sequences of IDRs are appended to various RNA-binding domains, containing RGG repeats (red rectangles). IDRs alone can drive phase separation, but RNA can further promote this process by interacting through RNA-binding domains. b) Phase separation of NICD requires multivalent counterions such as the positively charged partner. NICD contact one another indirectly through positive counterions (blue circles) that likely bind the negatively charged clusters along the contour of NICD and directly through aromatic/hydrophobic interactions. **Bottom panel:** Nuclear Pore complex (NPC). The central pore scaffold contains IDPs that are used for scaffolding in multiple ways. a) Linker Nups have long IDRs containing SLiMs (orange boxes) that interact with folded domains in other scaffold Nups to form distinct subcomplexes. b) Nup159, located on the cytoplasmic ring of the NPC, contain five tandem repeats of DID which recruit five Dyn2 homodimers to form the scaffold of the Nup82 subcomplex. The permeability barrier of the NPC is made up of intrinsically disordered FG Nups. Nuclear transporters (Kaps; in green) passage through the FG barrier via multivalent interactions with the FG motifs. **Top left panel:** Cytoskeleton. Actin nucleation/elongation factors are IDPs containing functional repeat motifs. a) Nucleation factor Spire contains four repeats of WH2 motifs (red boxes), each binding to an actin monomer (grey circles) to facilitate actin polymerization. b) Formins contain globular

FH2 domains (in turquoise) which bind actin monomers weakly and multiple polyproline repeats within its IDR regions which recognize profilin (orange circles) bound to actin to facilitate actin nucleation and elongation. Microtubule associated proteins (MAPS) are IDPs with repeats of short motifs (green or orange boxes) that bind the microtubule surface and tubulin dimers to stabilize or facilitate elongation of microtubules. Microtubules are made up of α/β tubulin heterodimers (purple and pink ovals) with disordered C-terminal tails that are decorated with PTMs (stars) that are recognized by different MAPs such as +Tip proteins.

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